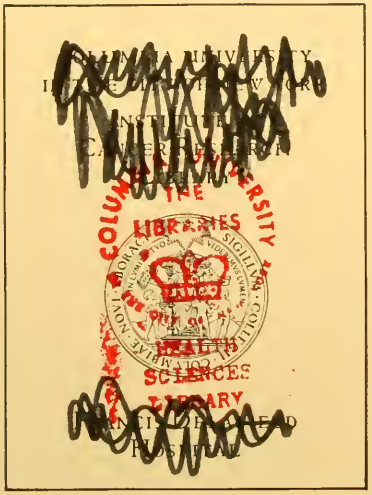

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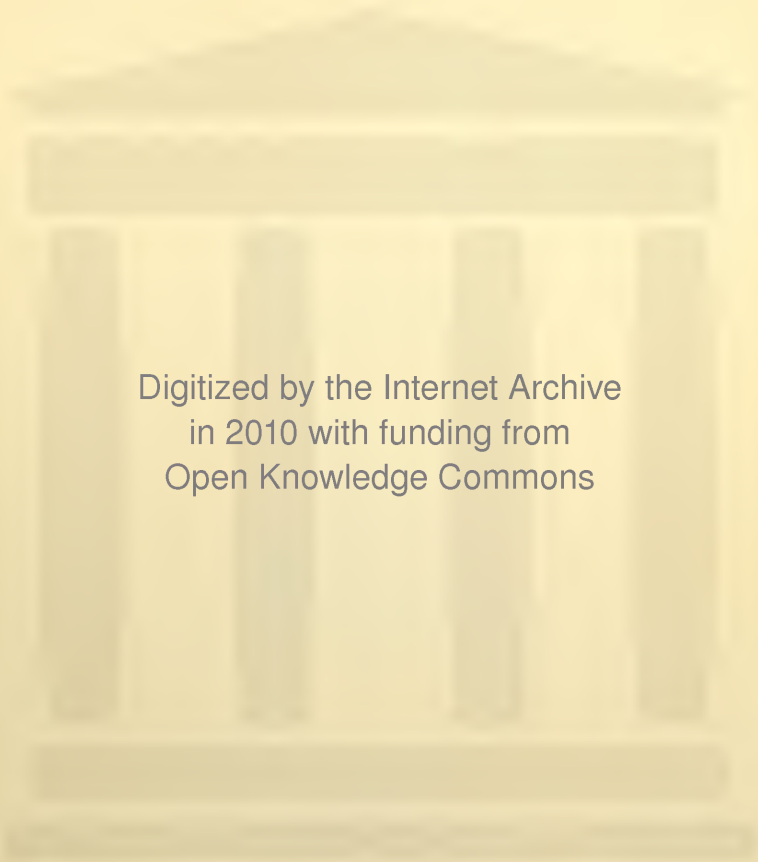

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SCIENTIFIC REPORT OF THE INSTITUTE OF CANCER RESEARCH

1951 — 1952

COLLEGE OF PHYSICIANS AND SURGEONS

COLUMBIA UNIVERSITY, NEW YORK

Scientific Report of the Institute of Cancer Research

1951 - 1952

College of Physicians and Surgeons

Columbia University, New York

Presented by the Cancer Research Coordinating Committee

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Introduction

Since this is our first formal Scientific Report, I shall take this opportunity to present the organization and philosophy of the Institute of Cancer Research to the agencies and individuals who provide financial support for the research work and to scientific institutions throughout the world with whom we are intellectually joined through the exchange of ideas and knowledge.

The Institute of Cancer Research was created in order better to integrate medical education, research, and patient care in the broad field of neoplastic disease. Laboratory research is conducted in a number of the departmental laboratories of the College of Physicians and Surgeons and in one hundred twenty-five laboratories contained in four stories of the College which are specifically allocated for cancer research. Clinical research on neoplastic disease and the medical care of cancer patients are accomplished on the wards of the Presbyterian Hospital and its affiliates, the New York Orthopedic Hospital, Babies Hospital, Sloan Hospital for Women, Squier Urological Clinic, and the Neurological Institute. These several units are closely linked both physically and with regard to their professional personnel. In 1951, an important addition was made to the Institute of Cancer Research, the 300-bed Francis Delafield Hospital, a modern clinic, hospital and research center, which was built by the City of New York. This institution is administered by the City of New York but is professionally staffed entirely by the Columbia University College of Physicians and Surgeons.

These constitute the physical facilities of the Institute of Cancer Research. More important than the space and equipment, however, are the investigators who teach, provide medical care for cancer patients and, through research, strive to broaden the knowledge of abnormal growth. The men and women who <u>are</u> the Institute of Cancer Research have academic

appointments in the Departments of the College of Physicians and Surgeons. Thus the Institute of Cancer Research implements the concept that investigation of the biological and clinical problems presented by abnormal growth can best be pursued by a voluntary interdepartmental cooperation. Within this organizational pattern the techniques, skills and specialized knowledge of multiple scientific disciplines can mingle and on a voluntary basis, form into ad hoc teams, and re-form as the issues under study dictate. The administrative mechanism to facilitate the work of this federation of scientists and clinicians is provided by the Cancer Research Coordinating Committee.

So much for the organization of the Institute of Cancer Research. What of its work? The body of this report deals almost exclusively with the research activities carried out during the year 1951-1952. Other functions of the Institute have not been neglected and I shall therefore briefly describe them here.

The educational program presented by members of the Institute of Cancer Research includes instruction of medical students through lectures, and clinical or laboratory research elective courses. Young physicians, dentists and nurses are trained in many aspects of human cancer through supervised experience on the wards of the Presbyterian and Delafield Hospitals. Post Graduate education is presented in a two-week intensive course at the Delafield Hospital and in teaching symposia in which members of the staff go as a unit to deliver lectures on various phases of cancer before medical groups in other communities. Finally the Institute recognizes its obligation to inform the public about cancer. An effort to meet this responsibility has been made through lectures, articles, radio and television programs.

The care of cancer patients can best be indicated by a few statistics. During the year 1951-52 4187 cancer patients were admitted to the

HOSPITALS OF THE COLUMBIA-PRESBYTERIAN MEDICAL CENTER AND 1100 CASES WERE ADMITTED TO THE NEWLY OPENED FRANCIS DELAFIELD HOSPITAL. THESE FIGURES SERVE AS A CONSTANT REMINDER OF THE MAGNITUDE OF THE PROBLEM PRESENTED BY NEOPLASTIC DISEASE AND AS A SPUR TOWARD FULL REALIZATION OF THE OPPORTUNITIES FOR ADVANCEMENT OF KNOWLEDGE OFFERED BY THE INSTITUTE OF CANCER RESEARCH.

JULY, 1952

ALFRED GELLHORN, M.D.

DEPARTMENT OF BIOCHEMISTRY

CANCER RESEARCH IN THE DEPARTMENT OF BIOCHEMISTRY HAS BEEN DEVELOPED FROM A BROAD BASE RANGING FROM THE CHEMISTRY OF NUCLEIC ACID IN WHEAT GERM TO CHOLESTEROL FORMATION IN HYPERCHOLESTEROLEMIC PATIENTS. IN ALL OF THE INVESTIGATIONS EMPHASIS HAS BEEN PLACED ON THE BASIC CHEMICAL PROPERTIES OF BIOLOGICALLY SIGNIFICANT CELLULAR COMPONENTS AND ON THE FUNDAMENTAL BIOCHEMICAL MECHANISMS IN CELL FUNCTION.

DR. ERWIN CHARGAFF AND HIS ASSOCIATES HAVE CARRIED ON PRODUCTIVE RESEARCH IN THE CHEMISTRY OF THE CELL. THEIR CHIEF FIELDS OF INVESTIGATION HAVE BEEN THE CHEMISTRY OF NUCLEIC ACIDS AND DESOXYRIBONUCLEOPROTEINS, LIPOPROTEINS AND THE CHEMISTRY AND BIOLOGICAL FUNCTION OF THE INOSITOLS. THESE ARE ALL CONCERNED WITH BIOCHEMICAL PROBLEMS FUNDAMENTAL TO CYTOLOGY, GENETICS, CANCER AND MANY OTHER FIELDS.

IN THE PAST YEAR, STUDIES ON THE DEGRADATION OF NUCLEIC ACIDS HAVE LED TO THE ISOLATION AND IDENTIFICATION OF AN APURINIC ACID. CHEMICAL CHARACTERIZATION OF LIPOPROTEINS HAVE YIELDED LIPIDS NOT BEFORE AVAILABLE FOR STUDY WHICH ARE NOW BEING IDENTIFIED CHEMICALLY. THE VITAMIN INOSITOL HAS BEEN STUDIED FROM VARIOUS STANDPOINTS. THE FUNCTION OF THIS CHEMICAL COMPOUND IS UNKNOWN. IN CERTAIN MICROORGANISMS IT IS ESSENTIAL FOR SURVIVAL. IN ORDER TO GAIN INSIGHT INTO ITS FUNCTION INVESTIGATIONS HAVE BEEN CARRIED OUT TO IDENTIFY THE MICROBIAL ENZYME SYSTEMS WHICH ARE CONCERNED WITH ITS DEGRADATION. OF PARTICULAR INTEREST HAS BEEN THE OBSERVATION THAT INOSITOL CAN PREVENT THE MITOTIC INHIBITION PRODUCED BY COLCHICINE ON THE ONION ROOT TIP. THIS STUDY HAS BEEN CONFIRMED BY DRS. CHARGAFF AND MURRAY USING MAMMALIAN CELLS GROWN IN TISSUE CULTURE AS THE TEST SYSTEM. INSIGHT INTO THE CHEMISTRY OF NUCLEIC ACIDS HAS BEEN EXTENDED THROUGH PRECISE CHARACTERIZATION OF THE DESOXYRIBOSENUCLEIC ACID OF WHEAT GERM. STUDIES ON THE ENZYMATIC DEGRADATION OF THIS COMPLEX MOLECULE HAVE REVEALED THAT THE "CORE" OF THIS NUCLEIC ACID IS DIFFERENT FROM THAT OF OTHER DEGRADED DNAS. THE COMPOSITION OF DESOXYRIBOSENUCLEIC ACID AND PENTOSE NUCLEIC ACID DURING THE EMBRYONIC DEVELOPEMENT OF SEA URCHIN EGGS HAS BEEN DETERMINED. SIMILARLY THE

NUCLEIC ACIDS FROM SALMON SPERM HAVE BEEN PRECISELY CHARACTERIZED. THE COMPARATIVE CHEMISTRY OF NUCLEIC ACIDS IS AN IMPORTANT STEP IN THE DETERMINATION OF THE CAUSES FOR SPECIES VARIATION.

Dr. Stephen Zamenhof working with Dr. Chargaff has studied the chemistry of the immunologically active substance of Hemophilus influenzae. In the past it has been believed that this substance or substances consist of polysaccharides. The work done in Dr. Zamenhof's laboratory during the past year has demonstrated that this is not the case, but rather that the antigenic material is a polyribosephosphate. In collaboration with Dr. H. Alexander, Dr. Zamenhof has worked on the chemistry of the transforming principle of H. influenzae. It has been shown that the principle is a desoxyribosenucleic acid. These investigations on microbial systems have far reaching implications in cancer research and may well point the way to developements of great significance in our understanding of cancer biology.

Dr. Z. Dische has continued his investigations on the mechanism of enzymatic synthesis of hexose-6-phosphate from ribose-5-phosphate. These studies are intimately connected with the general field of nucleic acid metabolism inasmuch as there is as yet no precise knowledge of the steps which take place in the formation and/or incorporation of the sugar moiety in the nucleic acid molecule. Dr. Dische has also worked on the chemical composition of the neutral mucopolysaccharides of human serum. These complex molecules may have significance in cancer inasmuch as there are alterations in the serum concentration of serum polysaccharides during the course of the disease. The nucleotides of the heart muscle under physiological and pathological conditions are also being actively investigated.

Dr. David Rittenberg and his colleagues have continued their studies on the rate of urea formation and excretion and of protein synthesis in man. A method has been developed for the determination of total body water using isotopically

TAGGED UREA. THE PHYSICO-CHEMICAL REACTIONS OF ENZYMES AND THEIR SUBSTRATES HAVE BEEN INVESTIGATED IN A VARIETY OF BIOLOGICAL SYSTEMS. IN COLLABORATIVE RESEARCH WITH THE DEPARTMENT OF MEDICINE, PROBLEMS IN HUMAN PHYSIOLOGY AND PATHOLOGY USING ISOTOPE-TRACER TECHNIQUES HAVE BEEN UNDER INVESTIGATION. THESE INCLUDED THE BILE PIGMENT METABOLISM IN POLYCYTHEMIA VERA AND IN THE NORMAL SUBJECT, THE LIFE SPAN OF ERYTHROCYTES IN THESE SAME INDIVIDUALS, AND CHOLESTEROL FORMATION IN HYPERCHOLESTEROLEMIC PATIENTS. PORPHYRIN SYNTHESIS IN CELL FREE SYSTEMS, AND THE IN VITRO FORMATION OF ANTIBODIES IN SPLEEN AND LIVER SLICES WERE ALSO INVESTIGATED.

DR. DAVID SHEMIN HAS DEMONSTRATED THE RELATIONSHIPS IN THE BIOSYNTHESIS OF PORPHYRINS TO THE TRICARBOXYLIC ACID CYCLE. HE HAS ISOLATED AN INTERMEDIATE OF THE KETOGLUTARATE--SUCCINATE REACTION WHICH IS THE PRECURSOR OF TWENTY-SIX CARBON ATOMS OF PROTOPORPHYRIN. THIS NEW SUCCINYL INTERMEDIATE NOT ONLY ARISES FROM KETOGLUTARATE BUT ALSO DIRECTLY FROM SUCCINATE. THIS CLEARLY DEMONSTRATES A PARTIAL REVERSIBILITY OF THE KETOGLUTARATE TO SUCCINATE REACTION, A PATHWAY WHICH HERETOFORE HAS BEEN CONSIDERED UNIDIRECTIONAL.

DR. DAVID SPRINSON AND HIS ASSOCIATES HAVE INVESTIGATED THE BIOSYNTHESIS OF SHIKIMIC ACID IN MUTANTS OF E. COLI WITH THE OBJECTIVE OF CHARACTERIZING THE PATHWAYS OF AROMATIC AMINO ACID METABOLISM. STUDIES IN MICROBIOLOGICAL SYSTEMS ON THE UTILIZATION OF METHYL GROUPS FROM GLYCINE AND SERINE IN THE SYNTHESIS OF CHOLINE AND PURINES HAVE CONTINUED. THE USE OF C^{14} LABELLED HISTIDINE FOR NUCLEIC ACID SYNTHESIS IN THE RAT HAS BEEN STUDIED. IT HAS BEEN FOUND THAT CARBON 2 OF THE HISTIDINE MOLECULE IS INCORPORATED INTO PURINES.

BIOCHEMISTRY LABORATORY, FRANCIS DELAFIELD HOSPITAL. THIS IS AN INTEGRATED UNIT UNDER THE DIRECTION OF DR. SAMUEL GRAFF WHICH IS PRIMARILY CONCERNED WITH NUCLEIC ACIDS AND CARCINOGENIC VIRUSES. DRS. ENGELMAN, GILLESPIE AND WEINTRAUB ARE INVOLVED IN THE SYNTHESIS OF NOVEL PURINE ANALOGS WHICH MIGHT EITHER FUNCTION AS PRECURSORS IN THE BIOSYNTHESIS OF THE NUCLEIC ACIDS, OR CONVERSELY, AS

INHIBITORS OF NUCLEIC ACID SYNTHESIS. ATTENTION HAS BEEN FOCUSSED ON ANALOGS OF GUANINE AND GUANAZOLO IN WHICH THE NITROGEN ATOMS OF THE PYRIMIDINE MOIETY OF THE PURINES HAVE BEEN REPLACED BY CARBON ATOMS. NINE NEW COMPOUNDS NOT PREVIOUSLY DESCRIBED HAVE BEEN PREPARED SO FAR IN THE COURSE OF THE WORK. THE BIOLOGICAL EVALUATION OF THESE COMPOUNDS IS IN PROGRESS.

THE PURINE ANALOGS AND DERIVATIVES ARE UNDER SYSTEMATIC PHYSICAL STUDY BY DR. MAY, UTILIZING PRINCIPALLY INFRA-RED ABSORPTION PHENOMENA IN AN ATTEMPT TO ELUCIDATE THE INTERNAL BONDING OF THE COMPOUNDS IN THE SEARCH FOR A CLUE TO THE CARCINOSTATIC BEHAVIOR OF GUANAZOLO AND TO THE BIOLOGICAL BEHAVIOR OF THE PURINES.

THE PHYSICAL CHARACTERISTICS OF NUCLEIC ACIDS AND NUCLEOPROTEINS FROM VARIOUS SOURCES ARE BEING DETERMINED BY DR. MAY IN AN ATTEMPT TO PARALLEL BIOLOGICAL DIVERSITY WITH PHYSICAL CONSTITUTION. HIS TOOLS ARE THE TISELIUS APPARATUS, THE ULTRACENTRIFUGE AND THE POLARISCOPE. OPTICAL PROPERTIES OF THESE COMPOUNDS HAVE NOT BEEN STUDIED ADEQUATELY IN THE PAST.

MRS. GRAFF IS ACTIVE IN THE PREPARATION, PURIFICATION AND CHEMICAL CHARACTERIZATION OF THESE NUCLEIC ACIDS. SHE HAS OBSERVED SOME INTERESTING DIFFERENCES IN THE CELLULAR ASSOCIATIONS AND BONDING OF THE NUCLEIC ACIDS AND IS PURSUING THIS PROBLEM FURTHER. SHE IS ALSO ENGAGED IN THE DIFFICULT STUDY OF THE POSSIBLY IMPORTANT CARBOHYDRATE MOIETY OF THE NUCLEIC ACIDS.

SINCE THE NUCLEIC ACIDS SEEM TO PLAY A ROLE IN THE CANCER PROBLEM AND ALSO IN EMBRYONIC DEVELOPEMENT, INDUCTION AND DIFFERENTIATION, AN INVESTIGATION USING VARIOUS NUCLEIC ACIDS IS IN PROGRESS UNDER DR. LIEDKE. THE RESULTS THUS FAR INDICATE THAT THE EFFECT ON THE DEVELOPEMENT OF FROG EMBRYOS AT DIFFERENT STAGES (BLASTULA TO TAIL BUD) WITH REGARD TO MORTALITY RATE AND ABNORMALITIES MAY POSSIBLY VARY, DEPENDING ON THE SOURCE OF NUCLEIC ACID. THE INHERENT RESISTANCE OF THE REACTING ORGANISM, HOWEVER, IS ALSO VERY IMPORTANT.

Implantations of diverse types of nucleic acids from different sources to competent ectoderm explants and the amphibian gastrocoel, have in a number of cases produced visible outgrowths of the reacting host ectoderm. An analysis of these outgrowths with regard to induction and cellular differentiation awaits further microscopic study.

Quantitative data is now being collected on the amount of RNA and DNA present in various germ layers and organ primordia where recent investigations seem to indicate definite relationships.

Depending on the results obtained it is proposed to continue this study by implantation of other nucleic acids and their derivatives and also to test germ layer and organ primordia in vitro under similar conditions.

Study of the immunological behavior of the mouse mammary carcinoma virus, with immediate emphasis on prophylaxis, continues. This phase of the work is largely under the direction of Dr. Heidelberger. The physical and chemical nature as well as the cultural characteristics of the virus are under study. Progress in these experiments and the nature of the problems are discussed in several recent publications cited in the bibliography.

This laboratory is heavily engaged in an investigation of the biochemistry of malignancy on a cytological rather than a histological level. This endeavor is made possible by our successful development of a technique for the isolation of discrete cellular components in sufficient yield, purity and nativity, for tracer studies. (See Bibliography) Dr. Margaret Marshall, is pursuing the application of this technique. She has in addition developed methods for the isolation of amino acids from the isolated particulate fractions. She is now ready for planned definitive tracer studies to compare the incorporation rates, not of whole cells, but of the individual cellular components, such as nuclei, mitochondria and microsomes from both normal and malignant tissues.

DEPARTMENT OF DERMATOLOGY

DR. HELEN O. CURTH HAS CONTINUED HER STUDIES ON ACANTHOSIS NIGRICANS AND ITS RELATIONSHIP TO CANCER. DURING THE PAST YEAR SEVERAL PATIENTS WITH ACANTHOSIS NIGRICANS WERE OBSERVED AND, AS WAS DONE BEFORE, MEMBERS OF THEIR FAMILIES WERE INTERVIEWED AND EXAMINED. ONE OF THE PATIENTS, A 66 YEAR OLD WOMAN (KRIEG), WHOSE DERMATOSIS HAS BEEN CONSIDERED AS BELONGING TO THE MALIGNANT TYPE, HAS NOT YET EXHIBITED ANY CANCER. SHE UNDERWENT AN EXPLORATORY LAPAROTOMY IN PRESBY-HOSPITAL EARLY IN JUNE, 1952 WITH NEGATIVE RESULTS. THE FACT, HOWEVER, THAT THE PATIENT SHOWED THE FIRST CUTANEOUS SIGNS OF ACANTHOSIS NIGRICANS AT THE AGE OF 65 AND THAT THE ERUPTION HAS BEEN PROGRESSING RAPIDLY POINTS TO A CANCER AS THE ACTIVATING PRINCIPLE OF HER DERMATOSIS. SIX OTHER MIDDLE-AGED PATIENTS WITH ACANTHOSIS NIGRICANS DIED; FOUR SUCCUMBED TO GASTRIC CANCER, ONE TO CANCER OF THE PANCREAS AND ONE TO CANCER OF THE GALL-BLADDER.

IN TWO OF THE PEDIGREES (STILES AND HALSVIK) OF PATIENTS WITH MALIGNANT ACANTHOSIS NIGRICANS CONSANGUINEOUS MARRIAGES OF THE FOREBEARS HAD OCCURRED SUGGESTING THAT WHATEVER FACTOR IS RESPONSIBLE FOR ACANTHOSIS NIGRICANS AND/OR CANCER MAY BE RECESSIVELY INHERITED.

IN GENERAL, IT CAN BE SAID THAT IT HAS BEEN NOTED THAT ALTHOUGH LITTLE INFORMATION OF FOREIGN ANCESTRY COULD BE OBTAINED, VERY OFTEN THE SAME GEOGRAPHIC ORIGIN AND ETHNOLOGICAL BACKGROUND EXISTED IN THE PATERNAL AND MATERNAL SIDES OF THE PATIENT'S FAMILY SO THAT A REMOTE RELATIONSHIP BETWEEN MEMBERS OF THE TWO SIDES IS NOT IMPOSSIBLE. IT WAS NOTED THAT ANCESTORS OF THREE PATIENTS (STRIECK, TINMAN AND KRIEG) CAME FROM GALICIA.

DURING THE YEAR ATTENTION HAS BEEN PAID TO SOME DERMATOSES OTHER THAN ACANTHOSIS NIGRICANS, WHICH ALSO HAVE BEEN ACCOMPANIED BY CANCER, SUCH AS DERMATOMYOSITIS AND DERMATITIS HERPETIFORMIS. IT WAS FELT THAT STUDY OF THE RELATIONSHIP BETWEEN CANCER AND THESE DERMATOSES MIGHT EVENTUALLY SHED LIGHT ALSO ON THE RELATIONSHIP BETWEEN CANCER AND ACANTHOSIS NIGRICANS. IT SOON BECAME APPARENT,

HOWEVER, THAT THE CANCERS ASSOCIATED WITH THESE VARIOUS DERMATOSES AS WELL AS THE MECHANISMS GOVERNING THEIR ASSOCIATIONS WITH CANCER ARE DIFFERENT FROM THOSE OF THE ASSOCIATION OF ACANTHOSIS NIGRICANS AND CANCER. FOR INSTANCE, IN PATIENTS WITH DERMATOMYOSITIS THERE IS A HIGH FREQUENCY OF CANCERS OF OVARIES AND BREASTS, OFTEN OF BOTH OVARIES AND BOTH BREASTS, WHILE IN MALIGNANT ACANTHOSIS NIGRICANS MAMMARY AND OVARIAN CANCERS RARELY OCCUR AND THEY HAVE NEVER BEEN OBSERVED BILATERALLY. NOT ALL CANCERS SEEN IN ASSOCIATION WITH DERMATOMYOSITIS ARE ADENOCARCINOMAS AS THEY ARE IN ACANTHOSIS NIGRICANS. FURTHERMORE, DERMATOMYOSITIS AND DERMATITIS HERPETIFORMIS SEEM DEPENDENT ON THE NEOPLASMS WHILE THE DEPENDENCE OF ACANTHOSIS NIGRICANS ON THE CANCER IS NOT PROVED.

Department of Medicine

Investigations in the Department of Medicine extend from basic theoretical studies for which there is no obvious immediate practical application to pragmatic clinical research of importance in the day to day care of cancer patients. The major philosophy guiding the overall research in this department is to extend the participation of Internal Medicine in the problems of Human Neoplastic disease. This accounts in part for the emphasis that is placed on basic and clinical research in the field of cancer chemotherapy and on the mechanism of anemia.

Dr. Joseph Turner has initiated studies on the nutrition of the micro-crustacean, Daphnia, with particular reference to hemoglobin metabolism. Elimination of the natural microflora of the intestine by the use of antibiotics appears to result in loss of reproductive activity in Daphnia, no doubt due to lack of an essential substance that is either liberated by bacterial action on food or synthesized from bacterial growth. It has been possible to subject individual Daphnia to the action of at least eight antibiotics in combination, or in succession, without destroying the animals. It is the hope that from this will develop a simple method for obtaining a "germ-free" population that will perhaps be generally applicable to the study of small aquatic animals.

Dr. Daniel Larson has studied the antibody responses following the administration of pneumococcus capsular polysaccharides in patients with Leukemia and allied disorders. Antibody determinations were carried out using the quantitative precipitin technique developed by Heidelberger. It was found that patients with chronic lymphatic leukemia and with multiple myeloma exhibited little or no antibody formation. Subjects with chronic myeloid leukemia showed antibody responses somewhat higher than the normal level. Individuals with acute leukemia demonstrated the production of exceedingly large amounts of antibody, and this response was impaired in

PATIENTS TREATED WITH CORTISONE. THUS IT IS APPARENT THAT THE LARGEST ANTIBODY RESPONSES CAN BEST BE CORRELATED WITH HIGH PERCENTAGE OF IMMATURE WHITE BLOOD CELLS.

DR. IRVING LONDON HAS CONTINUED WORK IN THE FIELDS OF PORPHYRIN, PROTEIN AND CHOLESTEROL METABOLISM. STUDIES ON THE ENZYMATIC MECHANISMS INVOLVED IN HEME SYNTHESIS HAVE BEEN FACILITATED BY THE FINDING THAT THIS PROCESS CAN BE DEMONSTRATED IN VITRO IN NON-INTACT IMMATURE MAMMALIAN AND AVIAN ERYTHROCYTE PREPARATIONS. THE OPTIMAL CONDITIONS FOR THIS SYNTHESIS, THE EFFECTS OF VARIOUS METABOLIC AGENTS SUCH AS FOLIC ACID, VITAMIN B_{12} AND LEAD, AND THE CELLULAR CONSTITUENTS CONCERNED WITH HEME SYNTHESIS ARE UNDER INVESTIGATION.

DR. LONDON'S PREVIOUS STUDIES ON THE ORIGINS OF BILE PIGMENT IN MAN HAVE SHOWN THAT A PORTION OF BILE PIGMENT IS DERIVED FROM ONE OR MORE SOURCES OTHER THAN THE HEMOGLOBIN OF MATURE CIRCULATING ERYTHROCYTES. AT PRESENT, INVESTIGATION INTO THESE ALTERNATIVE SOURCES OF BILE PIGMENT IS BEING PURSUED. FOR THIS PURPOSE, ISOTOPICALLY LABELLED PORPHYRIN COMPOUNDS WHICH ARE POTENTIAL PRECURSORS OF BILE PIGMENT ARE BEING PREPARED AND THEIR ROLE IN BILE PIGMENT METABOLISM EVALUATED.

STUDIES ON THE LIFE SPAN OF THE ERYTHROCYTE IN POLYCYTHEMIA VERA ARE BEING CONDUCTED TO DETERMINE WHETHER THE MARKED INCREASE IN ERYTHROCYTES IN THIS DISEASE IS ASSOCIATED WITH AN INCREASED RATE OF SYNTHESIS OF CELLS WITH A NORMAL LIFE SPAN. A CORRELATIVE STUDY ON THE METABOLISM OF BILE PIGMENT IS AIMED AT ELUCIDATING THE DISCREPANCY BETWEEN THE VERY HIGH ERYTHROCYTE LEVELS IN THE PERIPHERAL BLOOD AND THE RELATIVELY LOW BILE PIGMENT EXCRETION.

IN CONTINUING THE INVESTIGATION OF LIPID METABOLISM IN NORMAL AND PATHOLOGIC STATES, STUDIES ARE IN PROGRESS ON THE RATES OF TURN-OVER OF SERUM AND ERYTHROCYTE CHOLESTEROL AND PHOSPHOLIPID IN FAMILIAL HYPERCHOLESTEROLEMIA AND XANTHOMATOSIS. THESE STUDIES ARE CONDUCTED TO DETERMINE WHETHER THE INCREASED LIPID LEVELS ARE ASSOCIATED PRIMARILY WITH INCREASED SYNTHESIS OF THESE SUB-

STANCES OR WHETHER THERE IS A PRIMARY DEFICIENCY IN THE MECHANISMS RESPONSIBLE FOR THEIR ELIMINATION FROM THE BLOOD.

IN CONJUNCTION WITH DR. HELEN M. RANNEY, A STUDY OF THE CAPACITY OF VARIOUS TISSUES FOR ANTIBODY FORMATION HAS BEEN CONTINUED. BY SACRIFICING AN IMMUNIZED ANIMAL WHEN THE SERUM ANTIBODY TITER IS HIGH, AND WHEN ANTIBODY IS BEING SYNTHESIZED, IT IS POSSIBLE TO OBTAIN SURVIVING TISSUES CAPABLE OF ANTIBODY FORMATION IN VITRO. IF SUCH A TISSUE IS INCUBATED WITH AN ISOTOPICALLY LABELLED AMINO ACID, SOME OF THE AMINO ACID WILL BE INCORPORATED INTO THE NEWLY FORMED ANTIBODY PROTEIN. THUS THE PRESENCE OF SIGNIFICANT ISOTOPIC ACTIVITY IN THE ANTIBODY PROTEIN INDICATES ANTIBODY FORMATION BY THE TISSUE. THE DETERMINATION OF THE ISOTOPIC ACTIVITY OF A PASSIVELY ADMINISTERED ANTIBODY SERVES AS A CONTROL. WITH THIS TECHNIQUE SYNTHESIS OF ANTIBODY BY SPLEEN, BONE MARROW, LUNG, AND LIVER HAS BEEN DEMONSTRATED.

DR. RANNEY HAS UNDERTAKEN A STUDY OF THE SURVIVAL OF NORMAL TRANSFUSED ERYTHROCYTES IN PATIENTS WITH MYELOFIBROSIS AND MARKED SPLENOMEGALY. IN SOME OF THESE PATIENTS, ONLY SLIGHTLY DECREASED SURVIVAL OF TRANSFUSED ERYTHROCYTES HAS BEEN DEMONSTRATED, WHILE IN OTHERS, THERE HAS BEEN SIGNIFICANT DECREASE IN THE LIFE SPAN OF NORMAL RED BLOOD CELLS.

DR. MARCEL GOLDENBERG AND HIS ASSOCIATES, DRS. MAURICE M. RAPPORT AND IRVING SERLIN HAVE INVESTIGATED THE DIAGNOSIS OF PHEOCHROMOCYTOMA BY CHEMICAL METHODS. THEY HAVE FOUND THAT THE URINARY EXCRETION OF THE CATECHOL-AMINES, EPINEPHRINE AND NOREPINEPHRINE, CAN BE USED FOR DIAGNOSTIC PURPOSES. IN NORMAL MAN A TOTAL OF ABOUT 40 MICROGRAMS OF NOREPINEPHRINE IS EXCRETED DURING 24 HOURS; IN CASES OF PHEOCHROMOCYTOMA THE CATECHOL-AMINE EXCRETION IS INCREASED TO 200-2000 MICROGRAMS IN THE SAME TIME PERIOD. THIS APPLIES NOT ONLY TO CASES OF PHEOCHROMOCYTOMA WITH PERSISTENT HYPERTENSION BUT ALSO TO CASES WITH PAROXYSMAL HYPERTENSION.

IN THE LABORATORIES OF DR. ALFRED GELLHORN AND HIS ASSOCIATES, DR. ERICH HIRSCHBERG AND DR. JACOB KREAM, STUDIES HAVE BEEN CONDUCTED ON THE MECHANISM

OF ACTION OF CANCER CHEMOTHERAPEUTIC AGENTS WITH A VIEW TO THE DEVELOPMENT OF NEW AND PERHAPS BETTER OBJECTIVE CRITERIA FOR THE EVALUATION OF UNTRIED CHEMICAL COMPOUNDS. THESE INVESTIGATIONS, IN PART COMPLETED AND IN PART IN PROGRESS, WILL BE SUMMARIZED IN BRIEF.

IT WAS SHOWN THAT AZAGUANINE DEAMINASE, THE ENZYME WHICH CONVERTS THE CARCINOSTATIC AGENT, 8-AZAGUANINE, TO AN INACTIVE DERIVATIVE, 8-AZAXANTHINE, IS PRESENT IN THE CELLS OF NORMAL MOUSE TISSUES AND OF THREE TYPES OF 8-AZAGUANINE RESISTANT TUMORS IN A HIGHER CONCENTRATION THAN IN THE CELLS OF THREE OUT OF FOUR SEPARATE 8-AZAGUANINE SUSCEPTIBLE TUMORS. THIS STRONGLY SUGGESTS THAT THE ABILITY OR LACK OF ABILITY TO "DETOXIFY" THE DRUG IS AN IMPORTANT FACTOR IN DETERMINING THE FAILURE OR SUCCESS OF CHEMOTHERAPY WITH 8-AZAGUANINE. THESE ARE IN VITRO STUDIES.

AN INTERESTING AND POTENTIALLY SIGNIFICANT CORRELATION OF THE OBSERVATIONS DESCRIBED ABOVE WAS FOUND IN A COLLABORATIVE INVESTIGATION WITH DR. MARGARET MURRAY OF THE DEPARTMENT OF SURGERY AND DR. J. LAWRENCE POOL OF THE DEPARTMENT OF NEUROSURGERY. THE ACTIVITY OF AZAGUANINE DEAMINASE IN NORMAL BRAIN TISSUE AND IN THE MALIGNANT BRAIN TUMOR, GLIOBLASTOMA MULTIFORME, WAS DETERMINED. THE NORMAL BRAIN TISSUE HAS A HIGH CONCENTRATION OF THE ENZYME WHEREAS THE GLIOBLASTOMA HAS A NEGLIGIBLE CONCENTRATION OF THE ENZYME. THE GROWTH OF THE TUMOR IN TISSUE CULTURE IS INHIBITED BY 8-AZAGUANINE WHEREAS HUMAN FETAL BRAIN IS NOT INHIBITED TO THE SAME EXTENT. THESE FINDINGS SUGGEST A CLINICAL APPLICATION WHICH IS BEING EXPLORED NOW.

IN ORDER TO DETERMINE WHETHER THE IN VITRO OBSERVATIONS OF 8-AZAGUANINE DEAMINASE HAD RELEVANCE TO IN VIVO CONDITIONS, THE BLOOD LEVELS AND URINARY EXCRETION PATTERNS OF 8-AZAGUANINE AND 8-AZAXANTHINE WERE DETERMINED IN RABBITS AND DOGS AFTER THE ADMINISTRATION OF 8-AZAGUANINE. THE EXPERIMENTS DEMONSTRATED A RAPID DEAMINATION OF 8-AZAGUANINE.

A BROAD PROGRAM OF BIOCHEMICAL CHARACTERIZATION OF NORMAL AND NEOPLASTIC

TISSUES ON A CELL BASIS IS BEING CARRIED ON. EXTENSIVE DATA ON THE CELLULARITY AND ON THE NITROGEN, DESOXYRIBONUCLEIC ACID AND RIBONUCLEIC ACID CONTENT OF THE "AVERAGE CELL" OF A VARIETY OF NORMAL TISSUES OF THE MOUSE, RABBIT, RAT, DOG AND MAN AND OF EIGHT TRANSPLANTED EXPERIMENTAL ANIMAL TUMORS ARE NOW AVAILABLE. MOUSE AND RABBIT HEART MUSCLE, WHICH PRESENTS SOME SPECIAL PROBLEMS, IS BEING SUBJECTED TO PRELIMINARY ANALYSIS WITH Dr. MENARD GERTLER. PRELIMINARY DATA ON THE COMPARATIVE VITAMIN B_6 CONTENT OF NORMAL AND TUMOR TISSUE ARE BEING COLLECTED TO AID IN AN INTERPRETATION OF THE MECHANISM OF CARCINOSTASIS WITH DESOXYPYRIDOXINE.

MOST OF THE IMPORTANT CHEMOTHERAPEUTIC AGENTS EMPLOYED AGAINST NEOPLASMS APPEAR TO INTERFERE WITH NUCLEIC ACID METABOLISM IN SOME WAY. RECENT ISOTOPE DATA HAVE LED TO THE ASSUMPTION THAT THE SITE OF PRIMARY INHIBITION IS THE SYNTHESIS OF NUCLEIC ACID WHICH MUST PRECEDE CELL DIVISION. SEVERAL RELATED STUDIES IN THIS LABORATORY ON THE ACTUAL CELLULAR CONTENT OF NUCLEIC ACID OF TREATED AND UNTREATED TISSUES INDICATE THAT INHIBITION OF MITOSIS BY SOME OF THESE AGENTS IS NOT PRIMARILY MEDIATED THROUGH AN INHIBITION OF NUCLEIC ACID SYNTHESIS. WITH Dr. JOHN ULTMANN, IT HAS BEEN SHOWN THAT THE ADMINISTRATION OF A SINGLE DOSE OF NITROGEN MUSTARD TO RATS FOLLOWING PARTIAL HEPATECTOMY BRINGS ABOUT A STATISTICALLY SIGNIFICANT ACCUMULATION OF NUCLEIC ACIDS IN THE CELLS OF THE REGENERATING LIVER. EXPERIMENTS TO DETERMINE THE EFFECT OF CORTISONE IN THIS SYSTEM ARE IN PROGRESS.

THE EFFECT OF NITROGEN MUSTARD ON DESOXYRIBONUCLEASE AND RIBONUCLEASE (TWO ENZYMES WHICH MIGHT BE INVOLVED IN THE MECHANISM OF THIS ACCUMULATION OF NUCLEIC ACIDS) HAS BEEN STUDIED. ALTHOUGH THE DRUG INHIBITS THE ACTION OF CRYSTALLINE DESOXYRIBONUCLEASE, EVIDENCE HAS BEEN ACCUMULATED WHICH INDICATES THAT THIS DOES NOT OCCUR TO A SIGNIFICANT DEGREE IN THE LIVING TISSUES.

With Dr. Jane Gang the effect of cortisone on the nucleic acid content of two transplanted mouse tumors is being investigated. Results obtained to date point to an accumulation of nucleic acid in the tumor cells when the hormone inhibits growth.

8-azaguanine and aminopterin do not appear to bring about accumulation of nucleic acids in the cells of tumors which are inhibited by these drugs.

In a different approach to the mechanism of action of 8-azaguanine, experiments on the reversal of the action of this agent by various purines, nucleosides, and nucleotides have been carried out with the Brown-Pearce tumor grown in the anterior chamber of the rabbit eye. Guanine does not reverse the carcinostatic effect of 8-azaguanine, but adenine, adenosine, adenylic acid, guanosine, and guanylic acid do.

Medical Service of the Francis Delafield Hospital. The Francis Delafield Hospital is a 300 bed institution specifically dedicated to research on the problems of neoplastic disease. It is a part of the New York City Hospital system but is professionally staffed by physicians and scientists appointed by the Columbia University College of Physicians and Surgeons. The medical wards of this hospital provide an extraordinary opportunity to develop the contributions of internal medicine to cancer. The Medical Service not only fulfills the responsibilities of medical care to patients on its wards but also provides medical consultation service to the entire Hospital and operates the Admitting Clinic. The research program of the Medical Service is carried out in the Hospital wards and is integrated with laboratory research carried out in laboratories of the Hospital. Since the Francis Delafield Hospital has been in operation only since March, 1951, the major energies of the Medical Staff have been concerned with organization of the complex functions of a specialized hospital. The initial phase has now been completed so that more time will be available for research. Nevertheless a number of investigations

HAVE BEEN INITIATED AND RESULTS OBTAINED.

Dr. George Hyman has studied iron metabolism in patients treated with total body irradiation, nitrogen mustard and triethylene melamine, using tracer doses of radioactive iron. The results indicate that the radio-iron turnover rate is the most sensitive index of toxic effects of these agents on the hemopoietic system in man. Significant changes occur in this measurement long before there are detectable alterations of peripheral blood cellular elements or bone marrow morphology.

The transfusion of blood from patients with acute leukemia into suitable recipient volunteers with incurable advanced disease has been investigated in order to follow the peripheral blood changes and immune responses of the recipient (See Dr. Daniel Larson).

It was noted that the abnormal, blast forms disappeared within thirty minutes from the blood of the recipient and were never present in significant number. In addition, the studies to date have demonstrated a leukopenic response in the recipient occurring within the first 24 hours post-transfusion period. Attempts will be made to see if this holds true in a larger series and to elucidate the mechanism of this surprising finding. The transfusions were carried out in plastic bags and were given to the recipient immediately after phlebotomy in order to decrease the possible damage to white cells which had occurred in earlier studies by other investigators.

Dr. Daniel Larson has supervised experimental and clinical studies of a group of medical students on the effect of a chelating agent, ethylene diamine tetra-acetic acid (Versene) on serum calcium. Of particular interest was the demonstration that the chemical compound could reduce the serum calcium in hypercalcemic patients, but that the effect was temporary due to the rapid equilibrium between the skeletal and serum calcium.

Dr. Alfred Gellhorn and the resident medical staff including Dr. James Holland, Dr. Alexander Sahagian-Edwards and Dr. Nicholas Capeci have carried out a clinical evaluation of 1:4 dimethanesulfaneoxybutane (G.T. 41) which was kindly supplied by Dr. Alexander Haddow and his associates of the Chester Beatty Institute in London. The effect of the drug on a variety of neoplastic diseases has been investigated, but it has been found useful only in chronic myeloid leukemia.

The efficacy of a splenic extract, thrombocytosin, obtained from Johnson and Johnson Co., in thrombocytopenia was assessed. No therapeutic value could be determined.

Dr. Jack Davidson has carried out an exhaustive laboratory and clinical study on the rate of incorporation of radiophosphorus into nucleic acid mononucleotides. Using transplantable tumors in mice, Dr. Davidson found that the rate of incorporation was a sensitive measure of the effect of tumor inhibiting drugs. An attempt to apply the technique to the evaluation of potential drugs against human tumors does not appear to be feasible due to the practical difficulties presented by multiple biopsies and the variability of phosphorus incorporation into the same tumor located in different sites.

DEPARTMENT OF OBSTETRICS AND GYNECOLOGY

THE CANCER RESEARCH PROGRAM IN THE DEPARTMENT OF GYNECOLOGY MAY BE DIVIDED INTO THREE GENERAL SUBJECTS: STUDIES ON STEROIDS, HISTOCHEMICAL AND OTHER WORK ON MORPHOLOGY, AND A CONTROLLED INVESTIGATION OF CLINICAL THERAPY.

I. STUDIES IN THE CHEMISTRY AND METABOLISM OF THE STEROIDS.

DR. SEYMOUR LIEBERMAN IN THE CANCER INSTITUTE HAS BEEN WORKING ON FUNDAMENTAL ASPECTS OF THE SUBJECT, PARTLY IN THE DEVELOPEMENT OF METHODS, PARTLY IN THE WORK ON SPECIAL STEROIDS, PARTLY IN THE DETECTION AND MEASUREMENT OF URINARY STEROID METABOLITES.

A. INVESTIGATIONS IN THE NATURE OF THE STEROID METABOLITE EXCRETED IN THE URINE.

THE METHODS DEVELOPED FOR THE STUDY OF THESE SUBSTANCES HAS REQUIRED THE INVESTIGATIONS OF SEVERAL METHODS. 1. HYDROLYSIS OF THE STEROID GLUCURONIDATES NOW DEPENDS ON ENZYME HYDROLYSIS, USING BETAGLUCURONIDASE. IN ADDITION IT HAS BEEN SHOWN THAT CONTINUOUS ETHER EXTRACTION OF URINE ACIDIFIED TO 7N WITH RESPECT TO SULFURIC ACID IS A MOST EFFICIENT METHOD. ALTHOUGH FURTHER IMPROVEMENTS ARE BEING SOUGHT, THIS PROCEDURE COMBINING ENZYMATIC AND ACID METHODS SEEMS TO BE THE BEST THAT HAS BEEN DEVISED TO DATE. 2. CHROMATOGRAPHY REMAINS THE MOST IMPORTANT OF THE TECHNIQUES BY WHICH THE COMPLEX MIXTURE OF FREE STEROIDS ARE RESOLVED INTO THEIR INDIVIDUAL COMPONENTS. ONE STANDARDIZED PROCEDURE, USING 4 CHROMATOGRAMS HAS MADE POSSIBLE THE ISOLATION AND IDENTIFICATION OF 5 KETOSTEROIDS FROM THE URINE OF A PATIENT WITH AN OVARIAN TUMOR. EFFORTS ARE BEING MADE TO SIMPLIFY TECHNIQUES, SO THAT ONLY A SINGLE CHROMATOGRAM MAY BE NECESSARY AND A REASONABLY COMPLETE ANALYSIS OF THE URINARY KETOSTEROIDS MAY BE ACCOMPLISHED ON 500 CC OF URINE, IN ABOUT 10 TO 12 HOURS WORKING TIME. 3. RADIOMETRIC CHROMATOGRAPHIC METHODS ARE ALSO BEING STUDIED. THE APPLICATION OF CHROMATOGRAPHIC METHODS TO RADIO-ISOTOPICALLY LABELLED STEROIDS PROMISES TO PROVIDE A RAPID, EXTREMELY SENSITIVE TECHNIQUE, APPLICABLE TO ALL CLASSES OF STEROIDS. THE METHOD HAS BEEN USED, EMPLOYING LABELLED SUBSTANCES FOR ADSORPTION CHROMATOGRAPHY AND THE POSSIBILITIES OF PAPER CHROMATOGRAPHY ARE UNDER INVESTIGATION. 4. INFRA-RED

SPECTROSCOPIC METHODS HAVE BEEN ESTABLISHED IN THE LABORATORY DURING THE LAST YEAR MAKING IT POSSIBLE TO IDENTIFY UNEQUIVOCALLY AS LITTLE AS 25-100 MICROGRAMS OF A STEROID WHICH NEED NOT BE CRYSTALLINE. BY THESE TECHNIQUES IT IS POSSIBLE TO IDENTIFY 5 DIFFERENT KETOSTEROIDS IN A HALF DAY'S EXCRETION OF URINE. IT CAN BE USED ALSO TO OBTAIN VALUABLE INFORMATION ABOUT THE NATURE AND EVEN LOCATION IN THE STEROID NUCLEUS OF VARIOUS FUNCTIONAL GROUPS, SUCH AS THE CARBONYL AND HYDROXYL RADICALS. QUANTITATIVE MEASUREMENTS MAY ALSO BE MADE, WHICH FOR THE 17-KETO-STEROIDS APPEAR TO BE MORE ACCURATE THAN THE OLDER COLORIMETRIC ESTIMATIONS BY THE ZIMMERMANN REACTION.

B. OBSERVATIONS ON SPECIAL STEROIDS.

DURING THE COURSE OF THE YEARS WORK, A NUMBER OF INTERESTING OBSERVATIONS HAVE BEEN MADE AS BY-PRODUCTS OF THE MAIN INVESTIGATIONS.

A NEW STEROID, 1-ANDROSTENOLONE, WAS ISOLATED BY DINGEMANSE IN 1948 FROM A PATIENT WITH ADRENAL CARCINOMA AND IT WAS POSTULATED THAT THIS UNUSUALLY CONSTI-TUTED STEROID WAS CHARACTERISTIC OF ADRENAL DISORDERS AND WAS IN FACT THE PRECURSOR OF THE ALREADY WELL KNOWN, DEHYDROISOANDROSTERONE. DR. LIEBERMAN AND HIS GROUP HAVE DEMONSTRATED DURING THE LAST YEAR THAT 1-ANDROSTENOLONE WAS SIMPLY THE RESULT OF THE METHOD OF EXTRACTION, AND NOT A NATURAL PRODUCT, SINCE IT COULD BE OBTAINED WHENEVER AN AQUEOUS SOLUTION OF PURE DEHYDRO-ISOANDROSTERONE-SULFATE, COVERED WITH A LAYER OF BENZENE, WAS HEATED ON A STEAM BATH FOR 15 HOURS.

A NUMBER OF PREVIOUSLY, UNKNOWN SUBSTANCES, STEROID IMIDAZOLS, WERE PRO-DUCED IN THE LAST YEAR FROM STEROIDS POSSESSING KETOL SIDE CHAINS. THESE BASIC NITROGEN CONTAINING STEROIDS ARE INTERESTING FOR SEVERAL REASONS. THROUGH THEM A NEW AND MORE SPECIFIC METHOD FOR THE ESTIMATION OF CORTICOIDS MAY BE DEVISED, FOR CERTAIN STEROID IMIDAZOLS HAVE INTENSE ABSORPTION IN THE ULTRA VIOLET. FURTHER IT IS NOTEWORTHY THAT THE IMIDAZOL GROUP IS PRESENT IN SOME IMPORTANT BIOLOGICALLY ACTIVE COMPOUNDS, SUCH AS HISTAMINE, HISTIDINE, CARNOSINE, BIOTIN AND THE PURINES AND IT IS INTERESTING TO SPECULATE ABOUT THE BIOLOGICAL PROPERTIES THIS GROUP MAY

impart to various steroids. Preparations are being made to test these for their anti-hormonal activity.

C. Urinary Extracts Under Investigation.

The steroid excretion pattern in a number of special conditions is also being studied. 1. Progesterone as a Therapeutic Measure in Gynecologic Tumors. Progesterone in very large doses has been claimed to be beneficial in the treatment of various, gynecologic tumors, notably cancer of the cervix (Hertz). Daily doses of 150 mgms. of progesterone are being given to selected patients with cancer of the cervix for periods of time up to 42 days. All urine has been collected and is being studied to determine the chemical nature and quantities of the urinary metabolites and to seek out, if possible, a correlation between excretion patterns and clinical responses. Similar studies are being made using "oral progesterone" (pranone or ethynyl testosterone). 2. Metabolism of 17-ketosteroids. In collaboration with several other departments, studies are being made of the steroid excretion patterns after gonadectomy and adrenalectomy in patients with advanced cancer of breast or prostate. The urine of one male so treated has been found to contain no ketosteroids other than one metabolite of the cortisone administered to him. This "A-steroidal man" is being used to obtain information about various adrenal precursors. 3. Corticoids. As a part of this general program, a series of urines are being studied for their cortical metabolites. Work was begun on the complete urine collections of two women, during the last five months of their pregnancies and their first postpartum month. After determining the corticoids excreted by these normal pregnant women, the study will be extended to various other conditions, including certain tumors as well as certain complications of pregnancy.

The work in this laboratory has been fundamental both in its setting up of standardized procedures and in its establishment of various norms. From this solid basis the laboratory appears to promise steadily increasing productivity.

II. Studies in Adrenal Virilism.

Adrenal hyperplasia is accompanied by signs of virilism and by certain metabolic changes including the excessive excretion of 17-ketosteroids. It may be mistaken clinically for carcinoma of the adrenal and for "Cushing's syndrome". The laboratory of Dr. Joseph Jailer has been working on the differential diagnosis of these conditions and upon an elucidation of the special disturbance of adrenal physiology characteristic of each.

On the basis of data obtained on 15 patients with adrenal hyperplasia accompanied by virilism, it appears that the administration of cortisone results in a marked decrease in the urinary 17-ketosteroid excretion values to levels approximating normal for the age and sex of the individual. In contrast to this, two patients with the same syndrome but caused by adrenal carcinoma were similarly treated without any resultant change in the urinary 17-ketosteroid excretion values. Apparently, this response may be differentially used to diagnose the underlying pathology in this condition. The most plausible explanation is that the administration of cortisone inhibits the pituitary from secreting ACTH and thus the hyperplastic cells are inhibited whilst the adrenal carcinoma tissue is independent of pituitary control.

A similar situation exists in the Cushing's syndrome. Here again, cortisone administration resulted in a fall in the 17-ketosteroids in three patients with adrenal hyperplasia while it had no effect on a fourth patient who suffered from an adrenal carcinoma. In this latter patient, a hypophysectomy was performed subsequently without any demonstrable effect on the course of the disease. This tends to confirm the contention of the laboratory that adrenal carcinoma is independent of ACTH control while hyperplasia is not.

Further stimulation with ACTH of the hyperplastic adrenals in the virilizing syndrome has revealed certain basic metabolic differences in these glands from the normal and also from those of Cushing's syndrome. When ACTH is administered

TO PATIENTS WITH ADRENAL VIRILISM THERE IS NO SODIUM RETENTION, NO FALL IN CIRCULATING EOSINOLPHILS AND NO RISE IN CORTICOIDS AS CONTRASTED TO WHAT OBTAINS IN THE NORMAL OR IN THE CUSHING'S SYNDROME. THERE IS, HOWEVER, THE CHARACTERISTIC RISE IN 17-KETOSTEROIDS. THESE RESULTS TOGETHER WITH THE DATA OBTAINED AS A RESULT OF CORTISONE ADMINISTRATION HAS LED TO SPECULATION AS TO THE UNDERLYING BIOCHEMICAL AND ENZYMATIC DEFECT IN ADRENAL VIRILISM. IT IS BELIEVED THAT THE COMPOUND F PRECURSOR IS IMMEDIATELY DEGRADED TO A C_{19} STEROID (ANDROGEN) AS CONTRASTED TO THE NORMAL OR CUSHING'S SYNDROME WHERE THIS DOES NOT OCCUR TO ANY GREAT EXTENT. EXPERIMENTAL TECHNIQUES ARE BEING DEVELOPED TO TEST THIS SPECULATION WITH IN VITRO STUDIES.

III. STUDIES WITH ISOTOPICALLY LABELLED STEROIDS.

THE METABOLISM OF THE STEROIDS, ESPECIALLY THEIR LOCALIZATION IN VARIOUS PARTS OF THE BODY AND THEIR ROUTES OF EXCRETION, HAS CONTINUED TO BE THE SPECIAL FIELD OF WORK OF DR. GRAY TWOMBLY. THE PROGRAM HAS INVOLVED EFFORTS TO SYNTHESIZE CHEMICALLY VARIOUS LABELLED COMPOUNDS AND SECONDLY, TO UTILIZE THESE IN PHYSIOLOGIC STUDY.

LABELLED STILBESTEROL HAS BEEN SYNTHESIZED AND WORK WITH IT ON HUMAN SUBJECTS IS IN PROGRESS. EFFORTS HAVE ALSO BEEN MADE TO LABEL ESTRONE WITH C 14 AND IN PARTICULAR TO OBTAIN A MATERIAL, BY A SIMPLE METHOD AND WITH A HIGHER FINAL SPECIFIC ACTIVITY THAN THAT DEVELOPED BY HEARD AND HIS COLLABORATORS OF MONTREAL. THE FIRST STEPS HAVE PROVED DIFFICULT SO THAT INSUFFICIENT QUANTITIES OF THE DIMETHYL ESTER OF MARIANOLIC ACID HAVE BEEN OBTAINED. STUDIES WITH THIS OBJECTIVE ARE CONTINUING. RELATED WORK HAS BEEN GOING ON WITH THE CARCINOGENIC SUBSTANCE BETA NAPHTHYLAMINE, OF WHICH 17 MGMS. OF THE LABELLED MATERIAL HAS FINALLY BEEN OBTAINED. THIS HAS A SPECIFIC ACTIVITY OF 3.5 MICROCURIES PER MILLIGRAM AND IS LABELLED AT TWO POSITIONS (5 AND 8) IN THE MOLECULE. IT IS HOPED THAT THIS MATERIAL MAY AID IN DEMONSTRATING THE METABOLIC PATHWAYS OF THIS IMPORTANT HUMAN CARCINOGEN, ALTHOUGH ITS METABOLITES IN THE DOG AND RABBIT HAVE NOT YET BEEN IDENTIFIED.

The use of steroids in the therapy of advanced cancer of the breast is a well established practice, but the reasons for success in certain cases and complete failures in others remains a mystery. Labelled stilbesterol has been administered to 10 patients with breast cancer. These, however, have to date shown no gross differences in excretion rate, all returning about 40 per cent of the injected C 14 in the urine within 72 hours.

IV. Morphological Studies on Radiosensitivity of Cervical Cancer.

The great issue in the therapy of cancer of the cervix is between the adherents of the radiotherapeutic and the surgical methods. Reports from one particular source, and several others, have raised the possibility that cases susceptible to radiation may be distinguished on a practical basis from those that are resistant.

A series of biopsies are being taken from the growing edge of the tumor from patients in the course of x-ray or radium therapy. These sections are then studied to determine the changes in the nucleoprotein of the cells, as a measure of their reaction to radiation. Several techniques have been employed. Ultraviolet light microscopy, as developed by Caspersson seems to demonstrate two types of malignant cells, called A and B cells. The A cells are active with nuclei rich in desoxyribonucleic acid, while the B cells are regressing with nuclei poor in desoxyribonucleic acid and nucleoli rich in ribonucleic acid. Similar differences are demonstrable by a technique developed by the Italian workers, Barrigozzi and Dillepiane, who however make a smear preparation from a tissue crush and stain with acetocarmine. During the past year about 20 cases have been studied using these and other techniques to evaluate radiosensitivity. It is now believed that techniques have been perfected and a much expanded program is under preparation.

Although the problem of radiosensitivity now represents the central research project of Dr. Saul Gusberg's cytological laboratory, other work continues. In particular should be mentioned the continued survey of patients, with and without symptoms, by means of the Papanicolaou vaginal smear test for pre-clinical cancer

OF THE CERVIX AND THE CONSTANT EFFORT TO COLLECT AND EVALUATE THE CASES WITH INTRA-EPITHELIAL CANCER OF THE CERVIX.

V. Quantitative Study of the Functioning Elements of the Ovary in Cases of Gynecologic Tumor.

Abnormalities in the excretion of ovarian hormones have been alleged to be present in cases of several varieties of tumor and inhibition or stimulation of neoplastic growth has been observed when an estrogenic hormone is administered. Structural peculiarities of the ovaries have been reported to be present in animals with certain tumors. Reports on the ovaries of women with gynecologic tumors have been written in such general terms as to make the results largely meaningless.

A slow and painstaking study has been underway in this department in which it is believed that by the measurement of all structures related to the follicular apparatus in serial sections of known thickness the total functional capacity of the ovary can be measured. A preliminary report on the method has been published and the ovaries of a series of cases of cancer of the cervix and of some other tumors are being studied. This work is under the special direction of Dr. Howard Taylor.

In a related investigation, Dr. Earl Engle is examining qualitatively the histochemical characteristics of the ovary.

VI. A Controlled Study of the Therapy of Gynecologic Cancer.

Throughout the world there is indecision as to the relative value of surgery and radiation in the treatment of cervical cancer and as to the value of the addition of pre - or postoperative radiation to surgical excision in cases of cancer of the ovary, endometrium and vulva. To date there has been almost complete failure to find an answer to these controversies, partly at least because statistics of one type of therapy have had only for comparison those collected in a different institution or in a different five or ten year period. The differences in material, coming to different hospitals, or even to the same hospital in succeeding decades

ARE SO STRIKING AS TO MAKE COMPARISONS OF THERAPY, BY MEANS OF SERIES BASED ON SUCH DIVERGENT MATERIAL PRACTICALLY USELESS.

THE OPENING OF THE DELAFIELD HOSPITAL SEEMED TO OFFER OPPORTUNITIES TO DEVELOP A PROGRAM THAT MIGHT LEAD TO AN ANSWER TO SOME OF THESE MAJOR QUESTIONS IN GYNECOLOGICAL CANCER THERAPY. A COMMITTEE OF SEVEN MEMBERS, THREE RADIOTHERAPISTS AND FOUR GYNECOLOGISTS, HELD TWELVE MEETINGS AND DREW UP A PLAN FOR GYNECOLOGIC CANCER THERAPY. THIS, IN ORDER TO GET NUMBERS AS QUICKLY AS POSSIBLE, WAS TO BE APPLIED TO THE CANCER CASES OF BOTH THE PRESBYTERIAN AND THE DELAFIELD HOSPITALS.

THE BASIC PRINCIPLE ADOPTED WAS TO HAVE ALTERNATE TYPES OF TREATMENT AND TWO SIMULTANEOUS SERIES OF CASES, EACH UNDER THE DIRECTION OF THE SAME GROUP OF RADIOTHERAPISTS AND GYNECOLOGISTS AND IN THE SAME INSTITUTION. THE EXISTENCE OF TWO SUCH ALTERNATE TYPES OF THERAPY WAS DEEMED JUSTIFIED AS LONG AS THERE WAS ABSOLUTELY NO CONCLUSIVE OR RELIABLY SUGGESTIVE EVIDENCE THAT ONE METHOD OF TREATMENT OFFERED THE PATIENTS BETTER PROSPECTS THAN THE OTHER.

THE TYPES OF GYNECOLOGICAL CANCER WERE EACH DEFINED IN TERMS OF STAGES OF ADVANCEMENT AND FOR EACH VARIETY AND STAGE A STANDARD THERAPY WAS AGREED UPON. FOR NEARLY ALL VARIETIES AND FOR MANY SEPARATE STAGES TWO PLANS, BUT ONLY TWO, WERE AGREED UPON. THE MOST IMPORTANT PROBLEMS, CONCERNING THE MORE COMMON SITUATIONS, WERE AS FOLLOWS:-

CARCINOMA OF CERVIX, STAGES I AND II. THE TREATMENT IS TO BE BY IMMEDIATE RADICAL HYSTERECTOMY OR BY RADIATION THERAPY FOLLOWED IN THREE MONTHS BY LYMPH NODE DISSECTION.

CARCINOMA OF CERVIX, STAGES III AND IV. THE CONTRAST TO BE STUDIED IS THE RELATIVE PALLIATIVE EFFECTS OF CONTINUED RADIATION AND "ULTRA RADICAL" SURGERY.

CARCINOMA OF THE ENDOMETRIUM. THE POINT OF STUDY HERE IS THE RELATIVE ADVANTAGES OF PREOPERATIVE RADIATION.

Carcinoma of the Ovary. Here there are two problems, the value of post-operative X-ray in the favorable cases and the relative palliative effects of re-operation and of X-ray in the recurrent case.

This clinical program is evidently a long-term investigation. It is believed, however, that the therapy of the clinical material in gynecological cancer at the Medical Center is now so planned as to yield in the future the maximum of essential information that may lead to some reliable conclusions about therapy in this region.

Department of Radiology

A. Radiation Research.

The Radiation Research Laboratories are under the direction of Dr. G. Failla and Dr. Edith Quimby. The work of these investigators and their associates is primarily concerned with the physics of radiant energy and the application of this knowledge to clinical problems in radiotherapy.

Physics

The primary interest is the determination of the dose of ionizing radiations at any desired point or region in the biological system, in terms of the energy absorbed per gram of tissue. This is an essential prerequisite of all quantitative radiobiological studies. It is, unfortunately, a much more difficult problem than is generally realized.

In practice the determination of tissue dose (in ergs per gram) is always made by indirect means, but some methods are less direct than others. Also, some methods are simpler than others in special cases. It is, therefore, important to make a systematic study of the problem to develop suitable methods, to compare different methods and to ascertain their limitations.

Dosimetry of ionizing radiation may be simple or complicated depending on the point of view. If dose is defined as exposure of a biological object to a certain intensity of radiation for a certain length of time, the problem is very simple. Most of the biological work with radiation has been done in the past (and is being done today) on this basis. This is satisfactory for a qualitative exploration of the biological effects of radiation but not for the establishment of quantitative relationships and the study of the mechanism of radiobiological processes. For the latter purposes it is necessary to determine the dose in the region of the irradiated organism in which the radiobiological effect originates. For instance, in the study of the effect of radiation on the blood

COUNT, THE SIGNIFICANT DOSE IS THE ONE RECEIVED BY THE BLOOD-FORMING ORGANS, NOT THE DOSE RECEIVED BY THE CIRCULATING BLOOD. THEREFORE, THE DOSIMETRY PROBLEM IN THIS CASE IS TO DETERMINE THE DOSE IN THE BLOOD-FORMING ORGANS UNDER THE CONDITIONS OF EXPOSURE OF A PARTICULAR BIOLOGICAL EXPERIMENT - AND FOR MORE PRACTICAL PURPOSES UNDER THE CONDITIONS IN WHICH HUMANS ARE EXPOSED TO ALL KINDS OF RADIATION.

THE ABOVE EXAMPLE SERVES TO ILLUSTRATE ANOTHER IMPORTANT POINT. AT PRESENT THE DOSE IN THE BLOOD-FORMING ORGANS CAN BE ESTIMATED FAIRLY ACCURATELY UNDER CERTAIN CONDITIONS, BUT UNDER OTHER CONDITIONS THE UNCERTAINTY OF THE ESTIMATE MAY INVOLVE A FACTOR OF TWO OR THREE. IN THE PROTECTION PROBLEM THIS NECESSITATES THE INTRODUCTION OF ADDITIONAL FACTORS OF SAFETY. THIS IS SATISFACTORY UP TO A CERTAIN POINT. HOWEVER, AS THE KNOWLEDGE OF THE BIOLOGICAL EFFECTS OF RADIATION INCREASES AND THE PRESSURE FOR MORE "REALISTIC" PERMISSIBLE LEVELS INCREASES, IT WILL BE NECESSARY TO DETERMINE THE TISSUE DOSE IN THE BLOOD-FORMING ORGANS MORE ACCURATELY. THIS SITUATION IS TRUE IN GENERAL. THAT IS, THE DESIRABLE ACCURACY OF TISSUE DOSE DETERMINATIONS DEPENDS ON THE STATE OF THE SCIENCE AND ART AT A PARTICULAR TIME. OBVIOUSLY, IF THE DOSIMETRY PROBLEMS WERE SIMPLE THEY COULD BE WORKED OUT ONCE FOR ALL WITH SUFFICIENT ACCURACY FOR ALL BIOLOGICAL AND PRACTICAL PURPOSES. UNFORTUNATELY THIS IS NOT THE CASE AND THEREFORE WE MUST PROCEED ON THE BASIS OF SUCCESSIVELY MORE ACCURATE APPROXIMATIONS. THIS MEANS THAT WORK IN THIS FIELD WILL BE NECESSARY FOR AN INDEFINITE PERIOD OF TIME.

SOMETIMES IT IS POSSIBLE TO OBTAIN AN ACCURATE SOLUTION OF A PROBLEM UNDER SPECIAL CONDITIONS. AN EXAMPLE OF THIS IS THE DETERMINATION OF THE TISSUE DOSE IN A HOMOGENEOUS MEDIUM IN WHICH A BETA RAY EMITTING ISOTOPE IS UNIFORMLY DISTRIBUTED. THE METHOD AND APPARATUS WE HAVE DEVELOPED FOR THIS PURPOSE PERMIT AS HIGH ACCURACY AS MAY BE DESIRED FOR ANY PURPOSE. THE UNDERLINED WORDS INDICATE THE SPECIAL CONDITIONS. SINCE IN MANY PRACTICAL CASES (1) THE

TISSUE IS NOT HOMOGENEOUS, (2) THE ISOTOPE EMITS GAMMA RAYS AS WELL AS BETA RAYS AND (3) THE ISOTOPE IS NOT UNIFORMLY DISTRIBUTED IN THE ORGAN, TISSUE, OR CELL OF INTEREST, THE SOLUTION OF THE GENERAL PROBLEM IS FAR FROM COMPLETE. EVEN IN THE RELATIVELY SIMPLE SPECIAL CASE MUCH REMAINS TO BE DONE BECAUSE EXPERIMENTAL DATA MUST BE OBTAINED FOR DIFFERENT ISOTOPES OF PRACTICAL INTEREST. THE SAME THING APPLIES TO PRACTICALLY EVERY DOSAGE DETERMINATION PROBLEM.

THE PHYSICS PART OF THE WORK ON THE PROJECT IS DEVOTED ENTIRELY TO THE SOLUTION OF DOSIMETRY PROBLEMS. IN GENERAL, THE DIRECT APPROACH OF EXPERIMENTAL MEASUREMENT OF THE DOSE BY IONIZATION METHODS IS PREFERABLE. THIS IS NOT ALWAYS POSSIBLE, NOR IS IT ALWAYS DESIRABLE. THE MAIN OBJECT IS TO SOLVE A PROBLEM IN THE EASIEST WAY IN THE PRESENT STATE OF THE ART. IN ANY CASE IT IS IMPORTANT TO KNOW THE SIGNIFICANCE AND RELIABILITY OF THE RESULTS - WHICH SOMETIMES INVOLVES A GREAT DEAL OF ADDITIONAL EXPERIMENTAL WORK. THIS DOES NOT MEAN REPEATING THE SAME THING OVER AND OVER AGAIN, BUT CHECKING BY INDEPENDENT METHODS AND EXPERIMENTS.

SINCE THERE ARE MORE UNSOLVED PROBLEMS THAN CAN BE SOLVED IN THE FORESEEABLE FUTURE, A CHOICE MUST BE MADE AS TO THOSE TO BE ATTACKED FIRST. MANY FACTORS CONTROL THIS DECISION, INCLUDING THE SPECIAL APTITUDES OF THE LABORATORY PERSONNEL AT ANY PARTICULAR TIME. ALL THE PROBLEMS OUTLINED IN THE REPORT ARE RECEIVING SOME ATTENTION BUT THE EMPHASIS MAY CHANGE FROM TIME TO TIME.

Clinical Application

THE WORK CAN BE DIVIDED INTO TWO PARTS, THAT CARRIED OUT IN CONNECTION WITH THE RADIOTHERAPY DIVISION OF THE DEPARTMENT OF RADIOLOGY AND THAT IN CONNECTION WITH THE RADIO-ISOTOPE LABORATORY. IN BOTH CASES THE WORK IS MORE CLOSELY RELATED TO DEVELOPEMENT OF NEW METHODS IN CANCER THERAPY THAN IN FUNDAMENTAL CANCER RESEARCH.

1. RADIOTHERAPY:

A. ROTATION THERAPY. THE ROTATION CHAIR INSTALLED LAST YEAR HAS BEEN

EMPLOYED FOR TREATMENT OF A CONSIDERABLE NUMBER OF PATIENTS. UP TO THE PRESENT TIME A HORIZONTAL BEAM HAS BEEN EMPLOYED, THE PATIENT SITTING OR STANDING. METHODS FOR DOSAGE CALCULATION AND DOSAGE PLANNING HAVE BEEN IMPROVED, AND SPECIAL PHANTOMS HAVE BEEN CONSTRUCTED FOR EXPERIMENTAL CHECK ON THESE DETERMINATIONS. IT IS NOW PROPOSED TO EXTEND THE METHOD TO USE OF AN OBLIQUE BEAM, WITH THE PATIENT ROTATED WHILE LYING DOWN. IN THIS WAY A CONE OF RADIATION CAN BE DELIVERED, WITH ITS APEX AT THE POSITION OF THE TUMOR. METHODS FOR DOSAGE PLANNING AND CALCULATION ARE BEING DEVELOPED.

B. RADIUM DOSAGE. IN COLLABORATION WITH DR. MORTON KLIGERMAN A STUDY HAS BEEN COMPLETED ON THE DISTRIBUTION OF RADIATION DOSE THROUGHOUT THE HUMAN FEMALE PELVIS WITH DIFFERENT METHODS OF DISTRIBUTION OF RADIUM SOURCES. FROM THIS RESEARCH HAVE EMERGED RECOMMENDATIONS AS TO PREFERRED DISTRIBUTIONS AND STRENGTH OF SOURCES UNDER VARIOUS CLINICAL CONDITIONS.

DR. EDITH QUIMBY HAS COMPLETED A STUDY ON THE CALCULATION OF DOSAGE IN INTERSTITIAL RADIUM THERAPY. VARIOUS METHODS FOR DOSAGE CALCULATIONS HAVE BEEN PUBLISHED BEFORE. THE MOST COMPLETE AND CAREFUL ONE IS THAT OF PATERSON AND PARKER; IT IS BASED ON THE ASSORTMENT OF RADIUM NEEDLES COMMONLY AVAILABLE IN ENGLAND, WHICH ARE WEAKER THAN THOSE USUALLY FOUND IN THE UNITED STATES. MODIFICATIONS OF THE METHOD HAVE BEEN MADE TO FIT THE AMERICAN TYPE OF NEEDLES. THE EFFECTS DUE TO CERTAIN SHIFTS IN THE RECOMMENDED PATTERNS HAVE BEEN INVESTIGATED. RESULTS OF DOSAGE CALCULATIONS BASED ON THIS METHOD AND OTHERS HAVE BEEN COMPARED.

2. RADIO-ISOTOPE LABORATORY

A. RADIOACTIVE IODINE, I^{131}. A LARGE PART OF THE WORK WITH THIS ISOTOPE IS IN THE DIAGNOSIS OF THYROID FUNCTION AND HYPERTHYROIDISM. THE APPLICATION OF RADIO-IODINE TO THE DIAGNOSIS AND TREATMENT OF THYROID CANCER IS CARRIED OUT IN COOPERATION WITH A CLINICAL GROUP UNDER THE LEADERSHIP OF DR. VIRGINIA FRANTZ. ANY PATIENT TO BE OPERATED UPON FOR SUSPECTED THYROID CANCER RECEIVES A TRACER

DOSE OF I^{131} ABOUT 48 HOURS BEFORE THE OPERATION. IF CANCER IS FOUND, RADIO-AUTOGRAPHS ARE MADE TO DETERMINE WHETHER IT IS OF A TYPE WHICH TAKES UP RADIO-IODINE; THIS IS USEFUL INFORMATION IF METASTASES ARE PRESENT, AND IT IS DESIRABLE TO HAVE IT ON FILE EVEN IF NO METASTASES ARE FOUND AT THE TIME. IF TOTAL THYROIDECTOMY IS DONE, THE COMPLETENESS OF THE OPERATION CAN BE DETERMINED BY THE USE OF THE GEIGER COUNTER IN THE OPERATING ROOM. IF NO METASTASES ARE PRESENT AT THIS TIME NO OTHER ISOTOPE STUDY MAY BE DONE; ON THE OTHER HAND, TRACER TESTS ARE CARRIED OUT ANNUALLY, OR AT OTHER INTERVALS, IF THERE IS ANY INDICATION FOR THEM. IF METASTASES EXIST, THEY ARE STUDIED TO SEE WHETHER THEY CAN BE INDUCED TO TAKE UP IODINE IN SUFFICIENT AMOUNT TO PERMIT THERAPY. AN UPTAKE IN THE METASTASES OF FIVE PER CENT OR MORE OF THE ADMINISTERED DOSE IS AN INDICATION FOR TREATMENT. A NUMBER OF CASES HAVE BEEN TREATED, A FEW WITH EXCELLENT RESULTS.

b. RADIOACTIVE GOLD, Au^{198}. THIS SUBSTANCE IS USED PRINCIPALLY IN THE TREATMENT OF WIDESPREAD INTRAPERITONEAL OR INTRAPLEURAL METASTASES PRODUCING EFFUSIONS. THE ACTIVE MATERIAL IS INJECTED DIRECTLY INTO THE BODY CAVITY. THE WORK HAS NOT BEEN GOING ON LONG ENOUGH HERE TO PERMIT ANY EVALUATION OF RESULTS. TREATMENT IS UNDER THE COMBINED DIRECTION OF MEMBERS OF THE DEPARTMENTS OF SURGERY AND OF THERAPEUTIC RADIOLOGY. Miss Charlotte Schmidt IS IN CHARGE OF ADMINISTERING THE MATERIAL, AND HAS DEVELOPED A VERY SATISFACTORY METHOD FOR DOING THIS EFFICIENTLY AND SAFELY. SHE ALSO STUDIES THE DISTRIBUTION OF THE ACTIVE MATERIAL IN THE PATIENT FOR A FEW DAYS POST-TREATMENT.

c. RADIOACTIVE PHOSPHORUS, P^{32}. THIS IS USED IN THE TREATMENT OF LEUKEMIA AND OF POLYCYTHEMIA VERA, IN SELECTED CASES. IT IS ADMINISTERED UNDER THE DIRECTION OF THE THERAPEUTIC RADIOLOGISTS; THE ONLY FUNCTION OF THE ISOTOPE LABORATORY IS TO STANDARDIZE AND DISPENSE IT. NO RADIOACTIVITY MEASUREMENTS ARE MADE ON THESE PATIENTS.

Training Program

The quantitative study of the biological effects of ionizing radiations involves of necessity the accurate measurement of the tissue dose at the significant point or region in the irradiated organism. In general this requires a good knowledge of the characteristics of the different types of radiation, familiarity with highly specialized techniques, and considerable ingenuity. There are at present in this country very few people who are really competent to do this work. On the other hand the biological effects of ionizing radiations are being studied extensively in numerous laboratories. There is, therefore, a demand for physicists with this training and background, not only in A.E.C. installations but in universities and hospitals.

The measurement and safe handling of radio-isotopes are important considerations in connection with the use of these materials in biological and medical studies. Constant vigilance must be exercised and this requires proper training and long practical experience.

The Radiation Research Laboratory has specialized in these fields for many years and, therefore, it has a certain amount of responsibility to train new workers. In the past this has been done indirectly by training people for work within the laboratory and then helping them to get established elsewhere. This, of course, will continue, but in addition steps have been taken to accelerate and amplify the training program.

A program of studies, leading to the degree of Ph.D. in Biophysics has been set up at Columbia University. Biophysics is a much broader field than radiologic physics and radiobiology, which, however, are important branches thereof. A candidate for a Ph.D. degree in biophysics may specialize in either or both, depending on his background.

B. Radiotherapy

Dr. Morton Kligerman has continued to explore the clinical application of rotation radiotherapy. As described in the previous section he has collaborated with members of the Radiation Research Division on methods of practical dosage calculation in radium and x-ray therapy. Dr. Kligerman is also conducting a statistically controlled experiment on the use of dramamine and pyridoxine in radiation sickness. With Drs. John Robinson and George Fish of the Squier Urological Clinic, he has investigated the treatment of bladder cancer by segmental resection and radium implantation; preliminary results indicate that the combined approach is superior to either surgery or radiotherapy alone. Drs. Kligerman and Stuart Cosgriff have reported on the dramatic remission produced by adrenocorticotropic hormone in patients with symptomatic radiation fibrosis.

C. The Radiology Service at the Francis Delafield Hospital

1. Diagnosis

Dr. Arnold Bachman, chief of the x-ray diagnostic service, is conducting an active and interesting investigative program to extend the usefulness of x-ray in defining the extent of neoplastic disease. Studies in progress include the following:

1. Angiographic and venographic studies of tumors located in various parts of the body.

The vascular patterns of various tumors are being examined and studied in vivo and post morten in an attempt to delineate a characteristic and diagnostic appearance. It is felt that after sufficient information is obtained the method will prove of real value in localizing tumors and differentiating between benign and malignant processes.

2. Post-mortem portal venography and angiography.

The vascular appearance of the liver is being studied and the pattern of

HEPATIC METASTASES EXAMINED. IT IS HOPED THAT EVENTUALLY IN VIVO STUDIES OF THE VASCULAR PATTERN WILL BE OBTAINED. IN THIS MANNER LIVER METASTASES NOT APPRECIATED CLINICALLY MAY BE DISCOVERED.

3. TOMOGRAPHIC STUDIES, PARTICULARLY OF THE HEAD AND NECK, FOR THE DETERMINATION OF ABNORMALITIES AND EXTENT OF METASTASES. SPECIAL STUDIES OF THE TOMOGRAPHIC ANATOMY OF THE BASE OF THE SKULL ARE NOW BEING CARRIED OUT.

INVOLVEMENT OF THE BASE OF THE SKULL FROM MALIGNANCY OF THE HEAD AND NECK IS FREQUENTLY NOT APPRECIATED BY ROUTINE RADIOGRAPHIC EXAMINATION. WITH "BODY-SECTION" RADIOGRAPHY CERTAIN AREAS BECOME MORE CLEARLY DELINEATED. THIS MAY BE OF MAJOR SIGNIFICANCE FOR DETERMINING OPERABILITY AND EXTENT OF DISEASE. SINCE CERTAIN PHASES OF THIS WORK HAVE NOT BEEN PERFORMED PREVIOUSLY, IT BECOMES NECESSARY TO CLEARLY DEFINE THE NORMAL APPEARANCE.

II. THERAPY

DR. KENNETH LOEFFLER HAS INVESTIGATED THE EXCRETION OF C^{14} TAGGED POLYVINYL PYRROLIDONE WITH DR. JOHN SCUDDEN, DIRECTOR OF THE BLOOD BANK. POLYVINYL PYRROLIDONE (PVP) IS A SYNTHETIC HYDROCARBON WHICH HAS BEEN PROPOSED AS A PLASMA SUBSTITUTE. THE STUDIES TO DATE INDICATE THAT APPROXIMATELY 70 PER CENT OF THE RADIOCARBON INJECTED IS EXCRETED IN 24 HOURS. HISTOLOGICAL STUDY OF TISSUES OBTAINED AT POST MORTEM FAIL TO SHOW EVIDENCE OF MACROMOLECULAR STORAGE DISEASE. DR. LOEFFLER HAS ALSO COLLABORATED WITH DR. GEORGE HYMAN ON IRON METABOLISM STUDIES USING RADIOACTIVE IRON (SEE DEPARTMENT OF MEDICINE).

DR. VINCENT COLLINS AND DR. WARREN J. MEAD, PROFESSOR EMERITUS OF GEOLOGY AT THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY, HAVE MADE A VERY PRACTICAL APPLICATION OF THE PRINCIPLE OF DILATANCY. ACCORDING TO THIS PRINCIPLE, A GRANULAR MASS EXPANDS IF IT CHANGES SHAPE, OR IF EXPANSION IS PREVENTED, THE MASS RESISTS ANY CHANGE IN SHAPE. USING THIS PRINCIPLE A DEVICE HAS BEEN CONSTRUCTED WHICH HAS THREE APPLICATIONS IN THE RADIOTHERAPY DEPARTMENT: FIXATION OF THE PATIENT DURING TREATMENT, THE TAKING OF IMPRESSIONS FOR CASTS AND MOLDS, AND FOR USE AS

BOLUS MATERIAL. A REPORT OF THIS CLEVER AND USEFUL TECHNICAL ADJUNCT IN RADIOTHERAPY WILL APPEAR IN THE AMERICAN JOURNAL OF RADIOLOGY SHORTLY.

Drs. Vincent Collins, Carl Braestrup and Ruth Guttmann have devoted a major effort to the definition of the indications and limitations of super voltage radiotherapy with the two million volt X-ray machine. A systematic study of a variety of approaches to therapy has been made. These include:

A. Arrangement of fields in order to give the best tumor dose with least damage to surrounding organs.

B. The use of plaster contours to increase accuracy of measurement and treatment plan.

C. Calculation of tumor dosage in every instance.

D. The addition of a backpointer, pin and arc to the X-ray machine to add to the accuracy in lining up fields.

E. The use of a rotation chair for treatment of extensive deep seated tumors with the purpose of treating large areas with minimal damage to surrounding healthy tissues.

The application of these approaches in two million volt X-ray therapy provides certain definite improvements over the usual methods of radiotherapy. These include a better depth dose, less back scatter of radiation, less tissue absorption, less general radiation reaction and less skin reaction.

DEPARTMENT OF SURGERY

AS IN THE CASE OF OTHER CLINICAL DEPARTMENTS, CANCER RESEARCH IN THE DEPARTMENT OF SURGERY INCLUDES FUNDAMENTAL LABORATORY INVESTIGATIONS AS WELL AS APPLIED CLINICAL RESEARCH.

DR. CUSHMAN HAAGENSEN HAS CONTINUED RESEARCH ON THE PROBLEM OF MOUSE MAMMARY CARCINOMA. THE MAJOR EMPHASIS HAS BEEN ALONG TWO LINES:

1. DR. MICHAEL HEIDELBERGER HAS CONTINUED HIS STUDIES TO DEMONSTRATE IMMUNE REACTIONS TO THE AGENT. THE ANTIBODIES IN THE RABBIT SERUM PRODUCED BY INJECTION OF THE PURIFIED MOUSE CARCINOMA AGENT HAVE BEEN STUDIED QUANTITATIVELY. EXHAUSTIVE ABSORPTIONS OF ANTI-PARTICLE SERA BY MEANS OF C57 MILK PROTEINS AND BY C57 BREAST TISSUE EXTRACT LEFT A HIGH PROPORTION OF THE ANTIBODY NITROGEN STILL IN THE SERUM, SO THAT THE HIGHLY ABSORBED ANTISERA WERE STILL CAPABLE OF PRECIPITATING THE MOUSE MAMMARY CARCINOMA PARTICLES FROM SUSPENSION. THE ANTIBODY TO ANTIGEN RATIOS WERE LOW, OFTEN LESS THAN ONE. IN VIVO EXPERIMENTS TO TEST WHETHER OR NOT THE ANTIBODIES CAN NEUTRALIZE MOUSE MAMMARY CARCINOMA PARTICLES WHEN INJECTED INTO TEST MICE HAVE BEEN CARRIED OUT, BUT THE RESULTS WILL NOT BE APPARENT UNTIL THE MICE REACH OLD AGE IN ANOTHER YEAR. A REPORT OF THESE IMMUNOLOGICAL EXPERIMENTS HAS JUST BEEN PUBLISHED (JOUR. EXP. MED. APRIL, 1952)

2. DR. DAN MOORE HAS CONTINUED HIS EFFORTS TO PREPARE PURER PREPARATIONS OF THE AGENT FOR DEMONSTRATION WITH THE ELECTRON MICROSCOPE. HE HAS USED THE ANALYTICAL ULTRACENTRIFUGE AND THE ELECTROPHORESIS APPARATUS AS THE CHIEF MEANS OF SEPARATING OUT THE ACTIVE AGENT. HE HAS FOUND RECENTLY THAT TUMOR TISSUE IS A BETTER SOURCE OF THE AGENT THAN MILK, AND HAS SUCCEEDED IN PREPARING FROM THIS MATERIAL BETTER ELECTRON MICROSCOPE PICTURES THAN HAVE BEEN OBTAINED PREVIOUSLY IN ANY OTHER LABORATORY.

DR. MOORE, IN COLLABORATION WITH PROFESSORS PASSEY AND DMOCHOWSKI OF THE UNIVERSITY OF LEEDS, HAS CARRIED OUT TITRATION EXPERIMENTS WHICH HAVE SHOWN

THAT CARCINOMA IS PRODUCED IN MICE WITH DOSES OF THE AGENT AS LOW AS 10^{-7} MICROGRAMS OF NITROGEN. THIS PERMITS THE TENTATIVE CONCLUSION THAT THE NUMBER OF PARTICLES NECESSARY TO PRODUCE TUMORS IS OF THE ORDER OF HALF A MILLION.

IN CARRYING ON THESE EXPERIMENTS DURING THE PAST YEAR APPROXIMATELY 40,000 MICE FROM PURE BRED STOCKS OF C57 AND PARIS R III STRAINS HAVE BEEN USED. THE ANIMAL COLONY AT THE MOMENT HAS A POPULATION OF 5000, CONTAINED IN APPROXIMATELY 1500 CAGES. DURING THE PAST YEAR THE MOUSE DAIRY HAS PRODUCED APPROXIMATELY 3500 CC. OF MILK. MRS. EDITH COOLEY'S WORK CONCERNS THE STATISTICAL INVESTIGATION OF NEOPLASTIC DISEASE IN THE COLUMBIA-PRESBYTERIAN MEDICAL CENTER. SINCE MRS. COOLEY BECAME STATISTICIAN TO THE INSTITUTE OF CANCER RESEARCH, A METHOD OF SYSTEMATIC REVIEW OF THE HISTORIES OF ALL PATIENTS WITH NEOPLASMS COMING TO THE MEDICAL CENTER HAS BEEN ORGANIZED. FOR THIS PURPOSE A NEOPLASM SUMMARY CARD IS USED UPON WHICH THE DATA FOR EVERY NEOPLASM CAN BE RECORDED. THE RECORD OF HER FIRST FINDINGS HAS BEEN PUBLISHED IN THE "STATISTICAL INVESTIGATION OF NEOPLASTIC DISEASE" FIRST ANNUAL REPORT - 1948.

IN ADDITION, MRS. COOLEY HAS GIVEN STATISTICAL ADVICE OF GREAT VALUE REGARDING SEVERAL STUDIES OF MALIGNANT DISEASE THAT HAVE BEEN CARRIED ON RECENTLY IN THE DEPARTMENT OF SURGERY AND THE DEPARTMENT OF GYNECOLOGY.

AT PRESENT, MRS. COOLEY IS ALSO ENGAGED IN SUPERVISING THE ORGANIZATION OF A METHOD OF STATISTICAL INVESTIGATION OF NEOPLASMS IN DELAFIELD HOSPITAL WHICH WILL BE AN INVALUABLE AID TO MEMBERS OF THE STAFF WHO WISH TO USE THE RECORDS.

DR. DANIEL M. SHAPIRO HAS INITIATED A PROGRAM TO TEST THE VALIDITY OF THE HYPOTHESIS THAT AN APPROACH TO THE SELECTION OF CANCER CHEMOTHERAPEUTIC AGENTS LIES IN THE QUANTITATIVE BIOCHEMICAL DIFFERENCES BETWEEN NORMAL AND NEOPLASTIC CELLS. IT IS POSTULATED THAT SINCE METABOLITE INHIBITION BY AN

ANTIMETABOLITE DEPENDS UPON THE CONCENTRATION RATIO OF METABOLITE AND ANTAGONIST, THE FUNCTION OF, FOR EXAMPLE, A VITAMIN IN LOW CONCENTRATION IN CANCER TISSUE MIGHT BE BLOCKED BY MEANS OF AN ANTAGONIST WHILE ONLY MINIMALLY DEPRESSING THE METABOLIC ACTIVITIES OF THE SAME VITAMIN IN NON-MALIGNANT CELLS. THE CONCOMITANT ADMINISTRATION OF COMBINATIONS OF ANTAGONISTS, SELECTED UPON THE ABOVE BASIS, MIGHT RESULT IN SIMULTANEOUS INJURY TO MULTIPLE METABOLIC PATHWAYS WITHIN THE CANCER CELL.

DURING THE PAST YEAR EXPERIMENTAL CHEMOTHERAPY OF A TRANSPLANTABLE TUMOR IN MICE USING COMBINATIONS OF ANTI-TUMOR DRUGS AND VITAMIN ANALOGS HAS YIELDED THE FOLLOWING PRELIMINARY RESULTS.

(A) STILBESTEROL INHIBITS THE 755 BREAST CARCINOMA AND, IN COMBINATION WITH THE GUANINE ANTAGONIST 8-AZAGUANINE, PRODUCES A VERY MARKED TUMOR INHIBITORY EFFECT.

(B) IN ACCORD WITH THE MICROBIOLOGY SECTION FINDINGS THAT RIBOFLAVIN IS IN LOWER CONCENTRATION IN THE 755 TUMOR, FLAVOTIN, A RIBOFLAVIN ANALOG, INHIBITS THE GROWTH OF THIS TUMOR IN FEMALE MICE.

(C) TESTOSTERONE ENHANCES THE INHIBITORY EFFECTS OF THE PYRIDOXINE ANTAGONIST, DESOXYPYRIDOXINE TOXICITY.

(D) THE ADMINISTRATION OF PYRIDOXINE TOGETHER WITH DESOXYPYRIDOXINE PREVENTS THE USUAL CARCINOSTATIC EFFECT OF THE B_6 ANTAGONIST, THUS INDICATING ITS MECHANISM OF ACTION.

IN THE SURGICAL PATHOLOGY LABORATORIES, DR. JOHN PICKREN IS CARRYING ON A RESEARCH PROJECT ON THE INCIDENCE AND DISTRIBUTION OF METASTASES TO THE AXILLARY LYMPH NODES IN CASES OF CARCINOMA OF THE BREAST.

DR. ROBERT GRINNELL HAS CONTINUED HIS STUDIES ON THE DISTRIBUTION OF METASTASES FROM CARCINOMA OF THE RECTUM AND COLON. DR. GRINNELL IS ALSO CARRYING ON A STUDY OF THE EXTENT, FREQUENCY AND MECHANISM OF THE

DOWNWARD SPREAD OF CARCINOMA OF THE RECTUM IN THE WALL OF THE BOWEL.

THE WORK OF DR. RAFFAELO LATTES ON THE DIFFERENTIAL DIAGNOSIS BETWEEN GASTRIC ULCER AND NEOPLASM AND THE STUDIES OF DR. VIRGINIA FRANTZ ON RADIO-IODINE IN THYROID CANCER HAVE BEEN SUMMARIZED IN PUBLISHED ARTICLES (SEE BIBLIOGRAPHY).

DR. SAMUEL K. POWERS HAS DEVELOPED AN INSTRUMENT WHICH MAKES POSSIBLE THE STUDY OF THE CARDIO-VASCULAR RESPONSE TO MAJOR SURGERY. THE METHOD INVOLVES THE INJECTION OF SMALL AMOUNTS OF A RADIO-ACTIVE MATERIAL (Na^{24}) AND THE DETERMINATION OF THE RESULTING CONCENTRATION IN ARTERIAL BLOOD. THE METHOD IS SUITABLE FOR USE IN THE OPERATING ROOMS AND ON THE WARDS. FREQUENT MEASUREMENTS OF CARDIAC OUTPUT CAN BE CARRIED OUT IN THE PRE-OPERATIVE AND POST-OPERATIVE PERIOD.

THE EFFECTS OF MAJOR SURGERY, INCLUDING RADICAL CANCER SURGERY, HAVE BEEN STUDIED WITH SPECIAL REFERENCE TO THE RELATION BETWEEN BLOOD LOSS, CARDIAC OUTPUT AND SURGICAL TRAUMA. THE RESULTS FROM THESE STUDIES HAVE BEEN USED IN THE CLINICAL MANAGEMENT OF THOSE CASES FREQUENTLY AS A QUANTITATIVE GUIDE TO BLOOD REPLACEMENT.

THE EFFECT OF SPECIFIC DRUGS, SUCH AS DIGITALIS, EPHEDRINE, ETC. ARE BEING STUDIED IN THE MANAGEMENT OF SURGICAL TRAUMA.

DR. HUGH FITZPATRICK HAS UTILIZED SMALL PLASTIC CATHETERS AND LILLY CAPACITANCE MANOMETERS WITH A DIRECT WRITING OSCILLOGRAPH TO OBTAIN CONTINUOUS INTRAVASCULAR PRESSURE PULSE CURVES THROUGHOUT A VARIETY OF SURGICAL PROCEDURES. THE SMALL POLYVINYL CATHETERS ARE INTRODUCED THROUGH 20 GAUGE NEEDLES INTO ANY DESIRED PERIPHERAL ARTERY OR VEIN, INTO THE GREAT VESSELS OR INTO CARDIAC CHAMBERS. FROM ANY THREE SUCH SITES, ALONG WITH AN ELECTROCARDIOGRAM, SIMULTANEOUS AND DIRECT PULSE PRESSURE TRACINGS ARE OBTAINED.

THROUGHOUT RADICAL SURGICAL PROCEDURES FOR CANCER, THE EFFECTS OF ROUTINE MANIPULATIONS, COINCIDENT WITH ANY SPECIFIC OPERATION, EITHER BY THE SURGEON OR ANESTHESIOLOGIST, HAVE BEEN STUDIED. THESE STUDIES COMPLEMENT THE COOPERATIVE CONDUCT OF THE OPERATION, AS A CONTINUOUS AND DIRECT RECORD OF THE CIRCULATORY STATUS OF THE PATIENT IS OBTAINED AND THE EFFECTS OF EITHER BLOOD LOSS OR BLOOD REPLACEMENT, EITHER BY ARTERY OR VEIN, CAN BE FOLLOWED CLOSELY.

IN CASES OF CARCINOMA REQUIRING RADICAL NECK SURGERY, A CONTINUOUS RECORD HAS BEEN MADE ALSO OF THE INTRASPINAL FLUID PRESSURE AND THE EFFECTS OF PARTIAL OR COMPLETE OCCLUSION OF ONE OR BOTH INTERNAL JUGULAR VEINS HAVE BEEN QUANTITATED AS WELL AS THE GENERAL CIRCULATORY ADJUSTMENT THAT MAY OCCUR WITH SUCH PROCEDURES.

DR. MARGARET MURRAY HAS SUPERVISED THE RESEARCH OF A NUMBER OF COLLABORATIVE PROJECTS IN WHICH TISSUE CULTURE TECHNIQUES HAVE BEEN EMPLOYED:

A CHEMOTHERAPEUTIC AND METABOLIC STUDY OF HUMAN GLIOBLASTOMAS IN VITRO HAS BEEN PURSUED DURING THE PAST YEAR BY MRS. EDITH PETERSON, IN COLLABORATION WITH DR. J. L. POOL AND DR. ERICH HIRSCHBERG. THIS HAS INVOLVED THE INHIBITION OF THE CULTURED TUMOR TISSUE BY 8-AZAGUANINE, AND ITS PROTECTION BY GUANINE AND ADENOSINE, WITH CULTURED HUMAN FETAL GLIAL TISSUE AS CONTROL.

MRS. PETERSON HAS ALSO CONTINUED HER INVESTIGATION OF THE CIRCUMSTANCES SURROUNDING THE FORMATION OF MYELIN IN VITRO, AND HAS SHOWN THAT THE BEGINNING OF MYELIN-FORMATION (IN THE CHICK SENSORY GANGLION) IS COINCIDENTAL WITH THE MATURATION OF THE GANGLION CELL AS REGARDS FORM AND DISTRIBUTION OF NISSL SUBSTANCE. EVIDENCE HAS ALSO BEEN OBTAINED IN FAVOR OF THE PARTICIPATION OF THE SCHWANN CELL IN MYELIN FORMATION.

MRS. HELENA BENITEZ HAS CONTINUED HER STUDY OF MITOTIC POISONS, IN COLLABORATION WITH DR. E. CHARGAFF AND DR. HAWTHORNE. THIS HAS BEEN DIRECTED PARTICULARLY TOWARD INVESTIGATING THE RELATIONSHIP OF MESO-INOSITOL TO THE SPINDLE; AND DURING THE CURRENT YEAR IT HAS INVOLVED THE STUDY OF 3 COLCHICINE

ANALOGS (TROPOLONES) FOR THE FIRST TIME IN VITRO, AND THE DEMONSTRATION THAT THEY ARE NOT SPINDLE POISONS FOR RAT FIBROBLASTS.

MISS FRANCES MOTTRAM AND MISS ELIZABETH TADER, IN COLLABORATION WITH DR. A. P. STOUT AND DR. R. LATTES, HAVE CONTINUED TO STUDY AND RECORD THE CHARACTERISTICS EXHIBITED BY A VARIETY OF HUMAN NEOPLASMS IN TISSUE CULTURE, AS AN AID IN THEIR CLASSIFICATION AND DIAGNOSIS. WITH DR. FRED METTLER AND MR. STANLEY CRAIN THEY HAVE ENGAGED IN AN EFFORT TO RECORD ACTION POTENTIALS OF SINGLE NERVE AND MUSCLE CELLS IN VITRO. POTENTIALS HAVE SO FAR BEEN OBTAINED FROM CARDIAC AND SKELETAL MUSCLE CELLS.

DR. EDWARD L. HOWES HAS CARRIED OUT BIOLOGICAL STUDIES ON THE INFLUENCE OF ALTERED METABOLISM ON THE PROLIFERATION OF NORMAL EPITHELIAL CELLS, FIBROBLASTS AND BLOOD VESSELS AFTER A SINGLE INJURY UNDER THE INFLUENCE OF A PREVIOUSLY INSTITUTED METABOLIC ALTERATION. THESE RATES WERE COMPARED WITH THOSE OF MALIGNANT CELLS SUBJECTED TO THE SAME METABOLIC CHANGES. THE EXPERIMENTS WERE CONDUCTED IN RATS. RESULTS DURING THE PAST YEAR INDICATE THAT THE DEPRESSANT EFFECT OF CORTISONE ON MESENCHYMAL PROLIFERATION CAN BE FURTHER ENHANCED WHEN THE SERUM PROTEINS ARE LOWERED. THE CAPACITY OF ADENINE AND GUANINE TO OFFSET THE INHIBITION OF EPITHELIAL GROWTH CAUSED BY 8-AZAGUANINE HAS BEEN INVESTIGATED. IT HAS BEEN FOUND THAT ADENINE DEFINITELY PREVENTS THE ACTION OF 8-AZAGUANINE WHEN BOTH CHEMICAL COMPOUNDS ARE LOCALLY APPLIED.

DR. DAVID V. HABIF HAS CONDUCTED RESEARCH ON THE ALTERATION OF FLUID AND ELECTROLYTE SHIFTS IN PATIENTS UNDERGOING MAJOR SURGERY. THE INCREASING MAGNITUDE OF RADICAL CANCER SURGERY MAKES SUCH STUDIES OF PARAMOUNT IMPORTANCE. INCLUDED IN THE DETERMINATIONS ARE MEASUREMENTS OF EXTRACELLULAR VOLUME BOTH WITH INULIN AND SODIUM THIOSULFATE ALONG WITH TOTAL BODY WATER SPACE USING DEUTERIUM OXIDE. THE SODIUM SPACE MEASUREMENTS ARE CARRIED OUT WITH RADIOACTIVE SODIUM (NA^{24}).

This year's results demonstrate that one method of determining extra-cellular volume cannot be compared with another. It is impossible to be certain that extra-cellular fluid is being measured as such and this study resolves itself into a definition of just what is and is not being measured. It has been shown that the standard inulin method of a 6 hour constant infusion and 24 hour urine collection is not practical for use in surgical patients and that a 4 hour constant infusion taking into account urine loss during this period is not entirely reliable. The sodium thiosulfate method has given reproducible results and has been adopted as the method of choice.

The methods for determining extra-cellular and total body water along with 3 hour exchangeable sodium space are being used to evaluate quantitatively shifts of water and electrolytes in patients demonstrating marked alterations pre and post-operatively and those undergoing major surgery. Some 40 patients have been studied as regards total body potassium to evaluate quantitatively losses not reflected in the serum concentration and as a guide to the magnitude of change incident to major surgery.

Surgical Service of the Francis Delafield Hospital.

A laboratory and clinical study of the lymphatic circulation has been undertaken by Dr. David Ju. Dr. Ju has developed a method for accurately measuring the lymphatic circulation in the extremities of dogs using radioactive albumin. The method is now being applied to study various abnormalities in the lymphatic circulation of patients with cancer.

Drs. Joseph McDonald and Joseph Cairncross are directing special attention to the internal mammary node and supraclavicular node route of metastasis as an integral part of the broad study of breast cancer and its management. This involves biopsies of the lymph nodes from these areas in selected cases before treatment and biopsy of the internal mammary node in all cases undergoing mastectomy for cancer of the breast. As a corrolary to this, dissections of the

INTERNAL MAMMARY LYMPH NODES AT AUTOPSY ARE BEING DONE ON 100 CONSECUTIVE CASES IN AN EFFORT TO ASCERTAIN MORE ACCURATELY THE ANATOMICAL DISTRIBUTION OF THE LYMPH NODES IN THIS REGION. DR. MCDONALD IS CONDUCTING A LONG TERM PROGRAM TO IMPROVE THE CLINICAL APPLICATION OF PLASTIC AND RECONSTRUCTIVE SURGERY IN HEAD AND NECK CANCER. TEN MAJOR CASES OF THIS CATEGORY HAVE NOW BEEN COLLECTED, DOCUMENTED WITH MOTION PICTURE RECORDS AND FINE COLORED ILLUSTRATIONS PREPARED BY MR. IVAN SUMMERS, MEDICAL ARTIST.

NEUROLOGICAL SURGERY SERVICE.

DR. J. LAWRENCE POOL IS CONDUCTING A CLINICAL EVALUATION OF 8-AZAGUANINE IN THE TREATMENT OF GLIOBLASTOMA MULTIFORME (SEE DR. MARGARET MURRAY, DR. ERICH HIRSCHBERG).

DR. JAMES B. CAMPBELL IS ENGAGED IN DEVELOPING STEREOTOXIC MEANS OF INSERTING MEASURED AMOUNTS OF RADIOACTIVE MATERIAL FOR SPECIFIC PURPOSES IN DEEP SEATED BRAIN TUMORS.

DEPARTMENT OF UROLOGY AND THE UROLOGICAL SERVICE OF THE FRANCIS DELAFIELD HOSPITAL.

A HIGHLY INTEGRATED CLINICAL AND LABORATORY RESEARCH PROGRAM ON UROLOGICAL CANCER HAS BEEN INITIATED BY DR. PERRY HUDSON AND DR. JOHN REINER. THE TEAM WORKING UNDER THEIR DIRECTION CONSISTS OF CLINICIANS AND BIOCHEMISTS AND INCLUDES THE FOLLOWING INDIVIDUALS: DRS. GEORGE W. FISH, MORRIS LONDON, ALEX L. FINKLE, KENNETH TSUBOI, DONALD RAPPOPORT, HAROLD M. JOST, MARION BENTLEY, CASIMIR T. WOLAN AND MEIR PODBEREZEC.

A. CLINICAL RESEARCH AT THE FRANCIS DELAFIELD HOSPITAL.

ONE OF THE MAJOR CLINICAL PROJECTS UNDER STUDY DURING THE PAST YEAR HAS BEEN A COLLABORATIVE INVESTIGATION WITH THE MEDICAL SERVICE ON THE EFFECT OF BILATERAL ADRENALECTOMY ON CANCER OF THE PROSTATE, BREAST, BLADDER AND KIDNEY. AT THE PRESENT TIME EIGHTEEN PATIENTS HAVE BEEN STUDIED. TEMPORARY REMISSIONS HAVE BEEN INDUCED IN PATIENTS WITH DISSEMINATED PROSTATIC CANCER AND WHEN EXACERBATION OF THE DISEASE HAS OCCURRED, REINSTITUTION OF ESTROGEN THERAPY HAS BENEFITED THE PATIENTS WHO WERE PREVIOUSLY REFRACTORY TO ANDROGEN CONTROL THERAPY. SUBJECTIVE AND OBJECTIVE IMPROVEMENT HAS NOT BEEN NOTED IN PATIENTS WITH OTHER NEOPLASMS, BUT THE SERIES IS TOO SMALL TO PERMIT CONCLUSIONS NOW.

IN ADDITION TO THE EVALUATION OF THE EFFECT OF BILATERAL ADRENALECTOMY ON THE VARIOUS CANCERS, THE OPERATED PATIENTS HAVE ALSO BEEN STUDIED EXHAUSTIVELY FROM OTHER STANDPOINTS. KIDNEY FUNCTION STUDIES CONDUCTED BY DR. STANLEY BRADLEY USING PRECISE TECHNIQUES FOR THE DETERMINATION OF RENAL BLOOD FLOW, GLOMERULAR FILTRATION RATE AND THE FILTRATION FRACTION HAVE NOT SHOWN ANY IMPAIRMENT IN ADRENALECTOMIZED PATIENTS MAINTAINED ON CORTISONE. METABOLIC AND CARDIOVASCULAR STUDIES ON THE PATIENTS BEFORE AND AFTER ADRENALECTOMY HAVE ALSO NOT DEMONSTRATED ANY SIGNIFICANT CHANGES.

STEROID METABOLISM IS BEING CAREFULLY STUDIED IN THESE ADRENALECTOMIZED AND OFTEN ALSO GONADECTOMIZED INDIVIDUALS. BRIEF MENTION HAS ALREADY BEEN MADE IN AN EARLIER SECTION (SEE DR. SEYMOUR LIEBERMAN). RECENTLY, REPORTS HAVE APPEARED

IN THE EUROPEAN AND AMERICAN LITERATURE ON THE DESOXYCORTICOSTERONE--LIKE ACTIVITY OF GLYCYRRHIZINIC ACID. AN ATTEMPT WILL BE MADE TO CONFIRM THESE OBSERVATIONS IN POST-ADRENALECTOMY PATIENTS AND THE URINARY EXCRETION OF 17-KETOSTEROID AND NON-KETONIC STEROIDS WILL BE DETERMINED.

IN ANOTHER PHASE OF THE CLINICAL RESEARCH PROGRAM, 150 PATIENTS HAVE BEEN SUBJECTED TO A DETAILED UROLOGICAL EVALUATION WITH THE OBJECTIVE OF DETECTION OF EARLY CARCINOMA OF THE PROSTATE. IN 150 CONSECUTIVE PERINEAL PROSTATIC BIOPSIES, 30 PER CENT WERE FOUND TO SHOW CANCER. BY CORRELATING PRE-OPERATIVE DIAGNOSES WITH THE BIOPSY FINDINGS, IT HAS BEEN NOTED THAT APPROXIMATELY ONE HALF OF THOSE CASES FOUND SUITABLE FOR CURATIVE SURGERY WERE NOT SUSPECTED OF HARBORING A PROSTATIC CANCER PRIOR TO THE OPEN SURGICAL BIOPSY AND FROZEN SECTION PROCEDURES. ANCILLARY TO THIS STUDY A COMPARISON OF THE RELATIVE RELIABILITY OF TRANSURETHRAL AND PERINEAL PROSTATIC BIOPSY HAS BEEN MADE. THE AUTOPSY DATA WHICH HAS DEMONSTRATED THAT PROSTATIC CANCER ARISES PRIMARILY WITHIN THE POSTERIOR LOBE HAS BEEN CONFIRMED IN THE CLINICAL STUDIES AND PROVIDES A REASONABLE EXPLANATION FOR THE UNRELIABILITY OF TRANSURETHRAL BIOPSY IN EARLY DIAGNOSIS WHICH WAS FOUND.

A SYSTEM OF CLASSIFYING OR GROUPING PROSTATIC CANCER PATIENTS HAS BEEN DEVISED IN CONJUNCTION WITH Drs. A. P. Stout AND Edith Sproul OF THE PATHOLOGY SERVICE. THIS SYSTEM IS BASED UPON MICROSCOPICALLY ASCERTAINED EXTENT OF DISEASE, RATHER THAN UPON CYTOLOGIC OR HISTOLOGIC CRITERIA.

PROSTATIC CANCERS ARE PLACED IN FIVE DISTINCT GROUPS. GROUP I CANCERS ARE THOSE WHICH ARE STILL CONFINED TO THE SUBSTANCE OF THE GLAND. GROUP II CANCERS ARE THOSE IN WHICH TUMOR CELLS HAVE INVADED PERINEURAL LYMPHATIC SPACES, BUT NOT BEYOND THE ANATOMICAL CAPSULE OF THE PROSTATE. GROUP III CANCERS HAVE PROGRESSED AS FAR AS THE PERINEURAL LYMPHATICS OF THE GENITAL FASCIAL ENVELOPE SURROUNDING THE SEMINAL VESICLES. GROUP IV CANCERS ARE THOSE WHICH HAVE INVADED PERIPROSTATIC TISSUES. GROUP V CANCERS ARE THE FRANKLY METASTATIC TUMORS.

According to this classification, Groups I, II and III prostatic cancers are considered suitable for curative surgery. Groups IV and V are thought suitable for palliative therapy only.

This classification is similar to the League of Nations grading for cancer of the uterine cervix, and to Dukes' classification for neoplasms of the bowel. It seems likely that this system for grouping prostatic cancer will prove similarly useful in comparing results of therapy derived from various clinics; this has not previously been feasible, because there has not been general agreement on types or grades of prostatic cancer as seen by different pathologists.

A study of the value of prolonged, massive oral dosage of diethylstilbestrol in the management of metastatic prostatic cancer has been undertaken. Although the value of estrogen therapy is well established, the levels of dosage, particularly of stilbestrol have been controversial. In general, low dosages (1 - 5 mg. daily) have been employed. There are some studies also on the efficacy of intermediate dosage (up to 25 mg. daily). Until the present study, no attempts at thorough evaluation of massive estrogen dosage have been made. A total of 70 prostatic cancer patients have now been given a daily oral dose of 500 mg. of diethylstilbestrol. In many instances patients are now in their second year of medication. Some patients have received in a two year period more than 400 grams of the drug.

Immediate observations on these patients yield one clinically valuable fact: the incidence of undesirable side reactions (nausea, vomiting, painfully enlarged breasts, and dependent edema) approaches zero. It has, therefore, not been necessary to discontinue the medication because of drug idiosyncrasy.

Final effects of massive estrogen administration upon the neoplastic tissue cannot be determined at this time. There has been no grossly discernible difference, in this regard, between the various dosage levels.

In conjunction with androgen control therapy of prostatic cancer, an investigation has been initiated with Dr. Menard Gertler of the Medical Service on the serum lipids. It has been found that orchiectomy and the administration of estrogen is followed by a rise in the serum cholesterol and a greater rise in the serum phospholipids. The implications of these results in atherosclerosis are now being studied.

At the Francis Delafield Hospital a total of 200 patients (185 males and 15 females) who have undergone surgery on the Urological Service have been surveyed in a joint investigation with Dr. Gertler. These patients were studied from a cardiac viewpoint, in addition to the specific urological investigations, and were prepared pre-operatively to assure the best tolerance during surgery. A post-operative follow-up was done in each such case.

A preliminary survey indicates that cardiac complications have been kept to an unusually low frequency by this screening. Furthermore, it was demonstrated that patients with bundle branch block (right or left) were able to withstand with ease even major urological surgery.

Although the results have not been fully aggregated, it is obvious that numerous patients who are asymptomatic, are discovered to have potentially dangerous cardiac problems. The problems which may arise from such occult cardiac disease may be preventable if the pathology is recognized prior to operation. Furthermore, it has been learned that even severe cardiac patients withstand urological surgery well if proper cardiac medication and preparation is available pre-operatively.

B. Laboratory Research.

The laboratory investigative program is concerned with three major areas: the purification of prostatic acid phosphatase and study of its function, protein synthesis in the generative organs, and nucleic acid metabolism in normal and malignant cells of the genito-urinary system.

Excellent progress has been made in the isolation of the acid phosphatase from prostate glands. Using a variety of conventional and newly developed techniques, it has been possible to affect a thousand fold concentration of the enzyme. Properties of the enzyme are now under study.

Using acetone, calcium phosphate gel and ammonium sulfate fractions, sera from four prostatic cancer patients with high serum acid phosphatase values were fractionated. In all tests made, the serum enzyme proved to be identical with that found in non-cancerous prostates.

In the investigation of protein formation in generative organs, glutamine synthesis and the amide exchange reaction have been particularly emphasized. Experiments are also in progress to determine the feasability of using acid phosphatase formation as an indicator of net protein synthesis in surviving prostate slices. Changes in protein synthetic reactions are being followed during the process of aging and the influence of hormones upon these biochemical activities is being studied.

With Dr. Frederick Agate of the Department of Anatomy an investigation of cholesterol and ascorbic acid in the human adrenal is in progress. Within 45 minutes of the intravenous administration of ACTH, the cortical ascorbic acid decreases significantly suggesting an extraordinarily rapid effect of the pituitary hormone on adrenal steroid formation.

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George A. Hyman

SCIENTIFIC REPORT OF THE INSTITUTE OF CANCER RESEARCH

1952 — 1953

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Scientific Report of the Institute of Cancer Research

1952 - 1953

College of Physicians and Surgeons

Columbia University, New York

Presented by the Cancer Research Coordinating Committee:

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H. T. Clarke, D.Sc., Professor of Biochemistry
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Introduction

It is difficult to assess progress in cancer research from year to year particularly for those who are working directly in the field. The clinician who daily sees patients with neoplastic disease is constantly aware of the magnitude of the medical and social problem which is presented by cancer. The laboratory scientist who studies the physiological and biochemical phenomena of normal and tumor cells is impressed with the magnitude of the biological problem presented by neoplasia. Certainly every worker in the cancer field experiences periods of frustration and intense dissatisfaction with the apparently slow rate of progress toward the goal of effective cancer control.

It is recognized by all that the possibility exists that someone, somewhere, may make a revolutionary observation which will provide the key to the riddle of cancer. At the present time it seems highly unlikely that this will happen and few are willing to await this remote possibility with sublime patience. The vast majority prefer to press onward through investigations which cover broad areas utilizing the knowledge and techniques of all possible scientific disciplines.

The activities of the Institute of Cancer Research which are briefly described in the succeeding pages typify this latter approach. No claim can be made that an advance of vast significance has been achieved in the year 1952-53; knowledge, however, has been gained which coupled with that of other clinics and laboratories throughout the world must bring us closer to an understanding of the problems of abnormal growth.

The tremendous effort of the men and women of the Institute of Cancer Research during the past year has been made possible in large part by grants from a number of organizations and ultimately by the citizens of this country. It is with a feeling of gratitude and also a sense of cooperation that appreciative acknowledgment is made to:

The Albert and Mary Lasker Foundation

The Alma Toorock Memorial Fund

The American Cancer Society

The American Urological Research Foundation

The Atomic Energy Commission

The Committee on Growth, National Research Council

The Damon Runyon Memorial Fund

The Isabel McMillin Fund

The McAndrews-Forbes Company

The New York City Cancer Committee

The Pardee Foundation

The Schering Corporation

The United States Public Health Service

Alfred Gellhorn, M.D.

July, 1953

Department of Biochemistry

Dr. Erwin Chargaff and his group have continued active research in the field of Cell Chemistry. Although much of this work has been supported by funds not specifically designated for cancer research, practically all of his researches have a bearing on problems with cancer as can be seen from the titles of his publications during the academic year.

Dr. Stephen Zamenhof has continued his studies in collaboration with Dr. Hattie Alexander of the Department of Pediatrics on the influence of various agents on the transforming principle (heredity determinants, DNA) of Hemophilus influenzae and on human DNA. In particular, the action of carcinogenic agents was studied. It was found that the carcinogenic agents can be divided into two classes, those (such as urethane, carcinogenic hydrocarbons, carcinogenic azo dyes) which have no demonstrable effect on DNA in general, and the heredity determinants in particular, and those (such as nitrogen mustard, ultraviolet light) which even in minute amounts inactivate the heredity determinants.

Dr. Zacharias Dische has continued his work on mucopolysaccharides from blood and other tissues and has developed new and valuable color reactions for the detection and estimation of the heptoses, a class of carbohydrates which is now assuming considerable biochemical importance. These observations are now being applied to the study of mucopolysaccharides in the blood of patients with cancer.

Dr. David Rittenberg and his group have made important contributions to the theory of protein synthesis in humans, in intermediary metabolism of amino acids, and the physical-chemical aspects of enzymatic reactions.

Dr. David Shemin and his associates have continued their important studies on the mechanism of porphyrin formation and allied topics.

Dr. David B. Sprinson and his group have continued their studies of intermediary metabolism of threonine and histidine and have completed an investigation of the biosynthesis of shikimic acid by microorganisms.

Francis Delafield Hospital

The Biochemistry Laboratory of the Francis Delafield Hospital, a division of Columbia University's Department of Biochemistry, is engaged in a comprehensive program of study on fundamental biochemical aspects of malignant disease. The experiments are highly integrated and complementary and are the collaborative enterprise of the whole staff.

The model experiments on mammary carcinoma, culminating in the isolation from milk of the responsible virus, have entered a new phase after the demonstration that the isolated tumor-producing particles are immunologically distinct from normal milk components (Graff, Heidelberger and Haagensen; Heidelberger, Graff and Haagensen; Heidelberger, Leon, Graff and Haagensen). Propagation outside the animal body would be a valuable step forward. Promising experiments on in vitro cultivation of the virus are in progress under the combined efforts of Mrs. Ada Graff, Mr. Gerard Zagal, Mrs. Lucille Seguin (Department of Pathology), and Drs. Engelman and Graff.

The contrasting leukemia project established just a year ago, seeks to isolate and characterize the agent in the milk of old Storrs-Little mice which protects C58 mice from leukemia which is endemic in that strain. Mrs. F. J. Agate, Mr. Gerard Zagal, Mrs. Ada Graff, Dr. E. C. MacDowell and Dr. Graff have succeeded in establishing the several pure-bred strains in proper balance for milking and definitive fractionation experiments.

This laboratory has been engaged in the study of nucleic acids for a long time. The demonstration of the irreversible conversion of guanine to adenine in Tetrahymena geleii (Flavin and Engelman) which is the reverse of that in the metazoan suggested a possible bicyclic precursor for the two purines. Drs. Margaret Marshall, Engelman and Graff have subjected the question to experimentation wherein doubly labelled isotope experiments have proven that the purines are independently synthesized in the animal body without bicyclic intermediates. Further studies to trace the synthesis of the individual pyrimidine and imidazole moieties are in progress.

The finding by Graff and Barth in 1935 that the total purine content of the developing amphibian embryo is constant until beginning differentiation was amplified by Brachet, who concluded that desoxyribosenucleic acid undergoes synthesis at the expense of ribosenucleic acid in this system. Much later Villee, and later, Abrams, showed by means of isotopic markers that Brachet's conclusions were untenable. Experiments have therefore been started in this laboratory in an effort to rationalize the superficially contradictory evidence of the constancy of purines (common to both nucleic acids) and the obvious synthesis of desoxyribosenucleic acid in the developing embryo. Dr. Kathe B. Liedke, Mrs. Ada Graff and Drs. Engelman and Graff are comparing the isotopic labels of the individual purines at various stages of development of frog embryos specifically labelled in the nucleic acids. Confirmatory proof of original purine findings are being sought by Dr. David J. Smith who is partitioning the nucleic acid bases quantitatively by means of elution ion-exchange chromatography without previous isolation of the nucleic acids.

The amphibian embryo also provides an unique opportunity for the study of other chemical events occurring during simple cellular division. Drs. Marshall and Liedke are conducting precise amino acid analyses at progressive stages of embryonic development in a search for characteristic amino acid changes.

It is obvious that the essential amino acids are the ultimate precursor of the nitrogenous moieties of the nucleic acids in the metazoa. Drs. Engelman, Gillespie and Graff have therefore embarked on an investigation of the precise contribution to the nucleic acids of the various chain carbon atoms of the essential amino acids. The first of these, 2-C^{14}-histidine, has been prepared and is under experimental study.

A study of interference with essential cellular processes or growth inhibition not only provides experimental direction for growth studies but may eventually provide more or less specific chemotherapeutic agents against cancer. Drs. Engelman and Gillespie and Mr. Leonard Weintraub have been engaged in the synthesis

of compounds superficially related to guanine with these ends in view. Several new compounds, benzimidazoles and benzotriazole derivatives which have been found to inhibit growth in either T. geleii, Rana pipiens, or both, have been synthesized and are under detailed study by Dr. Liedke and Mr. Sheldon Greer (Department of Zoology).

Dr. Leopold May is investigating the molecular structure of these groups by means of infrared analysis in a systematic manner in an effort to arrive at some relation between structure and biological action.

This laboratory is engaged in a collaborative study with the Department of Medicine in the Francis Delafield Hospital under the direction of Dr. A. Gellhorn on amino acid metabolism in cancer. The research involves application of intermediary metabolic tracer methods. N^{15}-labelled aspartic acid has been administered to a leukemic patient. Incorporation and transfer of the amino group in the serum proteins, white cells, and urine are under investigation by Dr. Donald Tschudy, Mrs. Graff and Dr. Graff. The application of these procedures to the study of solid tumors is anticipated.

Dr. Sumner Levine has been concerned with the role of intracellular structures in protein metabolism. This work involves a consideration of phosphorylation mechanisms and their role in biosynthetic processes. Dr. Levine has also been active in the study of substrate bonds, especially those involving carcinogens. He has evolved a theory for the multipoint binding of substrate to protein and is engaged in experimental applications of this theory.

Department of Medicine

As in previous years, much of the research of Dr. Alfred Gellhorn and his associates, Dr. Erich Hirschberg, Miss Alice Kells and Marie Hanson, has been devoted to studies along two general lines: a) the biological evaluation of potential carcinostatic agents in a variety of experimental neoplasms; and b) the elucidation of biochemical mechanisms of action of active tumor-inhibiting compounds. A number of studies of different nature have also been initiated and will be discussed briefly.

a) Screening Experiments - A series of benzimidazoles and benzotriazoles synthesized by Dr. C. T. Bahner of Carson-Newman College were tested against mouse mammary adenocarcinoma 755 and Sarcoma 180 with consistently negative results. No inhibition of these tumors was seen with a number of other unrelated agents, including hexestrol and chloroquine.

The cross-linking agent 1,4-dimethanesulfonyloxybutane (GT 41, Myleran) was tested extensively in the laboratory, in conjunction with a program of clinical evaluation in man. This drug, at a daily dosage level of 10 - 25 mg./kg., effectively inhibited the growth of mouse mammary adenocarcinomas 755 and Eo771 and of the Brown-Pearce squamous-cell carcinoma in rabbits; mammary adenocarcinoma RC and Sarcoma 180 were inhibited in some experiments, but the drug was without effect on lymphosarcoma 6C3HED and myelogenous leukemia C1498.

Three new tumors have been added to the spectrum of neoplasms available for chemotherapeutic evaluation of compounds: mouse gliomas 26 and C3H19 and the ascitic form of lymphosarcoma 6C3HED.

These laboratories are participating in a collaborative experiment sponsored by the American Cancer Society in which the effect of a representative number of chemotherapeutic agents in a wide variety of biological and biochemical systems is being compared and evaluated. As part of this program, the carcinostatic effect of these agents against the Brown-Pearce tumor in rabbits and mammary adenocarcinoma 755 and gliomas 26 and C3H19 in mice is under investigation.

b) Mechanism of Action - Following the demonstration that tumors susceptible to carcinostasis by 8-azaguanine are low or lacking in an enzyme which deaminates and thus inactivates the drug, it had been shown that human glioblastoma exhibits negligible azaguanine deaminase activity whereas normal adult brain has an active deaminase system. In view of the possible clinical application of this finding, attempts were made in collaboration with Dr. J. Lawrence Pool of the Department of Neurosurgery to ascertain the blood levels of 8-azaguanine in man after the administration of the highest tolerated dose of the drug. Daily doses above 3 - 5 mg./kg. were not tolerated well; these levels contrast with the doses required in rabbits (25 mg./kg.) or mice (50 mg./kg.) for consistent tumor inhibition. A method was worked out for deproteinizing serum by ultrafiltration which permitted analysis of undiluted human serum for very small amounts of 8-azaguanine by filter paper chromatography and spectrophotometry. The accuracy of the method was sufficient to show that the level of the drug in the blood of patients given a dose of 3 - 5 mg./kg. was negligible even a few minutes after the injection.

In another series of experiments with 8-azaguanine, carried out in collaboration with Dr. Thelma Flint and Dr. Margaret Murray of the Department of Surgery, it was demonstrated that tissue cultures of the Brown-Pearce tumor, which were severely damaged by low levels of 8-azaguanine, were devoid of appreciable azaguanine deaminase activity. This finding is analogous to the earlier studies with human glioblastoma in tissue culture and further supports the correlation between susceptibility of a tumor to this drug and its ability to inactivate it by enzymatic deamination.

A collaborative investigation of the effect of a series of other chemotherapeutic agents on brain tumors in vivo and in vitro is being carried out with Dr. Margaret Murray and Mrs. Edith Peterson.

In another approach to the mechanism of action of carcinostatic agents, further work has been done to establish the effect of various agents on the nucleic acid content of the cells of some experimental neoplasms and of regenerating rat liver.

It has been reported that nitrogen mustard, when administered to rats at the beginning of liver restoration induced by partial hepatectomy, does not interfere with the synthesis of new nucleic acid but rather brings about a transitory accumulation of both nucleic acids in the liver cells. An analogous study with cortisone in this biological system has been completed with Dr. Stanley Einhorn. This drug acts in a different way from nitrogen mustard, since it was shown that inhibition of the formation of new cells during the first week of liver restoration keeps pace with and appears to depend on the inhibition of the de novo synthesis of nucleic acids by cortisone.

A series of experiments has been devoted to a study of the effect of cortisone on the nucleic acid content of the cells of mammary adenocarcinoma 755 and Sarcoma 180. The data obtained with cortisone are consistent with the idea that this agent may inhibit both the synthesis and the utilization of nucleic acids; experiments are in progress to define this problem further. 1,4-Dimethanesulfonyloxybutane, which by isotope measurements has been shown to have no effect on nucleic acid synthesis (cf. Dr. J. D. Davidson), brings about the expected accumulation of nucleic acids in the cells of tumor 755 under the proper conditions.

A more precise definition of the locus or loci of action of various carcinostatic agents on nucleic acid metabolism may be envisaged by a combination and correlation of the "dynamic" (i.e. precursor incorporation studies) and "static" (i.e. determination of actual nucleic acid content of cells) approaches to this problem. In collaboration with Dr. J. D. Davidson of Delafield Hospital, preliminary experiments have been carried out to evaluate the feasibility of such a joint program.

In conjunction with Dr. John Ultmann of New York Hospital, preliminary experiments were undertaken to assess the effect of x-ray therapy of chronic lymphocytic leukemia on the nucleic acid content of the leucocytes. In two patients, a decrease of the white blood cell count from 270,000 - 570,000 to 8,000 - 20,000 following treatment was accompanied by a significant increase in the RNA/DNA ratio of the leucocytes. The significance of this finding cannot be assessed at the present time.

c) Other studies -- The possible cocarcinogenic activity of tobacco tar is being studied in experiments in which the skin of mice is painted with benzene, croton oil, or tobacco tar following a few applications of benzpyrene, an epithelial carcinogen. These long-term experiments are not complete enough to permit any interpretation.

As part of a continuing program of biochemical characterization of normal and neoplastic cells, a series of nucleic acid determinations were carried out on four lines of the ascitic form of lymphosarcoma 6C3HED, two of which were started in these laboratories and two of which were provided by Dr. T. S. Hauschka of the Philadelphia Institute of Cancer Research. The values for DNA/cell were consistently lower in one of the Philadelphia lines than in the three other lines; this difference is in general agreement with data on nuclear fragmentation and lobation obtained by Dr. Hauschka.

In addition to the biological and biochemical studies using normal and neoplastic mammalian tissues, microbiological systems have been employed during the past year in other research problems.

Recent interest has been focused on phenylbutazone (Butazolidin), an antipyretic, anti-inflammatory, and analgesic agent with certain physiological similarities to cortisone. In view of a report that cortisone was able to replace the citrovorum factor for Leuconostoc citrovorum, experiments were carried out to establish whether phenylbutazone shared this property of cortisone. In these experiments, neither cortisone nor phenylbutazone were able to replace citrovorum factor, but the latter caused an inhibition of growth of the microorganisms. Experiments are in progress to determine the mechanism of this bacteriostatic effect. These observations may have some relevance to the known hematopoietic toxicity of the drug in man.

Most studies in neoplasia, including the ones reported here, are focused on some aspect of the rapid proliferation of cancer cells. Another characteristic of these cells, their relative lack of differentiation, has not been subjected to extensive experimental study, principally owing to the scarcity of adequate test

materials. In earlier studies, the slime mold Dictyostelium discoideum was shown to be a suitable test organism for studies on differentiation, in view of the essential separation of proliferative and morphogenetic processes in its life cycle. As part of the collaborative experiment sponsored by the American Cancer Society which was alluded to earlier, the effect of the series of chemotherapeutic agents chosen for all the studies in this program is being evaluated on several stages of differentiation and morphogenesis in this organism. This series of experiments will serve as the first step for an attempt to elucidate the biochemical mechanisms of differentiation in the absence of cell division and proliferation.

Dr. Emily N. Loeb and Dr. Abbie I. Knowlton, in conjunction with Dr. Herbert Stoerk of the Merck Institute for Therapeutic Research, have studied the effects of several adrenal steroids on the growth of the adrenalectomized rat while maintained on a sodium-restricted diet. With desoxycorticosterone acetate (DCA) a straight-line correlation between growth and the logarithm of the dose obtained over a wide dosage range, whereas no dose of cortisone acetate (CA) was found which encouraged growth. With combinations of the two steroids, the growth-promoting effects of DCA predominated until doses of CA in excess of 0.32 milligrams per day were reached. Above this level growth was inhibited and with further increase in CA dosage actual weight loss occurred in spite of the administration of large amounts of DCA concomitantly.

Another aspect of the relation of adrenal steroids to growth which has been studied is the reported ability of the addition of liver powder in the diet to counteract the suppressive effect of cortisone acetate upon growth. It has been established that this beneficial effect is observed only when the steroid is administered orally, and that no effect is demonstrable when the hormone is injected.

Dr. Marcel Goldenberg, together with Dr. Irving Serlin and Mrs. Theodora Edwards have developed a rapid chemical screening method for pheochromocytoma. The urinary excretion of catecholamines in 16 cases of pheochromocytoma was correlated with the clinical picture.

A method for chemical quantitation of noradrenalin and adrenalin has been developed. Together with Dr. Jan P. Mijnssen the influence of dopa decarboxylation inhibitors on the catecholamine content of the adrenal medulla has been studied in rats.

Medical Service of the Francis Delafield Hospital

Dr. Jack D. Davidson and Miss Elizabeth Freeman concluded studies on the in vivo incorporation of radiophosphorus into the nucleic acids of human tumors obtained at operation. The results were disappointing, for the lack of homogeneity of human tumor tissue, the AEC restrictions on administering very active radioactive phosphorus, and the unpredictable variability in time between the administration of the tracer ion and the availability of the tissue specimen all combined to invalidate the majority of the experiments conducted on 22 patients. The project was far from sterile, however, because valuable information was obtained on analytical methods, and the technical hazards will be helpful to other investigators contemplating comparable studies.

Dr. Davidson has developed the techniques for isolating nucleic acids from small samples of tissue and has utilized these in dynamic studies of DNA and RNA synthesis in experimental tumors. His attention has been particularly directed toward studying the effect of chemotherapeutic agents on this essential biochemical synthetic process. Experiments with 8-azaguanine have demonstrated that, associated with tumor inhibition, there is a significant depression of the incorporation of P^{32} into DNA and RNA. In a companion series of experiments with 1,4-dimethanesulfonyloxybutane (GT 41, Myleran), although the 755 mammary carcinoma was inhibited by the drug, no change was observed in the rate of incorporation of phosphorus into the nucleic acids. These studies are being expanded to include a large number of biologically active chemical agents and also do determine the effects of these drugs on the incorporation of other nucleic acid precursors.

In preparation for the experiments on the incorporation of glycine and formate which will be undertaken, Dr. Davidson has done considerable work on methods for

the separation of nucleotides. An ion-exchange technique was worked out using the formate form of Dowex-1 resin columns. A by-product of these studies was the development of an automatic fraction collecting machine which cuts fractions on the basis of their optical density to ultraviolet light.

Dr. James Holland, together with Dr. Alex Sahagian-Edwards and Miss Ethel Danielson, carried out a series of studies on patients with symptomatic hypercalcemia due to osteolytic metastases. This syndrome carries an ominous prognosis and available therapeutic measures are often of little avail. Dr. Holland and his associates utilized the chelating agent ethylene diamine tetra acetic acid as a means of sequestrating the excessive circulating ionic calcium. They demonstrated that following intravenous injection it was possible to reduce the serum calcium to normal levels. Unfortunately, in hypercalcemia due to this cause additional calcium is mobilized from the skeleton, and within hours, hypercalcemia recurs. These investigators concluded that EDTA was not therapeutically useful but their observations added information on the biology of hypercalcemia in metastatic cancer.

Dr. Alfred Gellhorn and Dr. James Holland, with the assistance of Drs. Daniel Choy, Charles Crandall and Arthur Snyder, evaluated the new steroid, dihydrotestosterone (Androstanolone, Neodrol) in the therapy of disseminated carcinoma of the breast. They concluded that this drug offered no significant advantages over testosterone propionate.

Dr. Alex Sahagian-Edwards reviewed the frequency of adrenal metastases in various neoplastic diseases. He found that approximately 50 per cent of autopsied cases of bronchogenic carcinoma had metastases in the adrenal glands. The importance of this observation was highlighted during the year by the demonstration of classical Addison's disease in two patients with bronchogenic carcinoma. Dr. Sahagian-Edwards has emphasized that this complication of bronchogenic carcinoma may become increasingly significant as radiotherapeutic methods for the control of the primary tumor are improved.

Dr. Elliott Osserman has initiated studies on paper electrophoresis of serum proteins in patients with neoplastic disease. He has already demonstrated the value

of the method in the diagnosis of Bence-Jones protein in the serum and urine of patients with multiple myeloma.

Dr. Donald Tschudy has started experiments on amino acid nitrogen utilization in patients with cancer. Using N^{15}-labelled aspartic acid it will be possible to assess the rate of serum protein and tumor protein synthesis in selected cases.

Dr. Alfred Gellhorn and Dr. James Holland completed a clinical evaluation of 1,4-dimethanesulfonyloxybutane (GT 41, Myleran) in a variety of neoplastic diseases including carcinoma of the breast, lung, colon and pancreas, multiple myeloma, and chronic myelogenous leukemia. Only in the latter disease was a favorable response observed.

Dr. George Hyman, together with other members of the Medical Staff, has studied the effect of 6-mercaptopurine in acute leukemia, multiple myeloma and reticulum cell lymphosarcoma. It is encouraging that the drug appears to have a significant effect in the latter disease since the response to other available chemotherapeutic agents is unsatisfactory. Acute leukemia in children also responds to the drug as described by Burchenal (cf. also Wolff), but the course of multiple myeloma does not seem to be modified.

Dr. George Hyman is directing a comprehensive investigation of the mechanism of anemia in patients with neoplastic disease. During the past year he has demonstrated that in patients with disseminated epithelial tumors hemolysis is an important mechanism of anemia. The rate of red cell destruction is comparable to that previously described by Ross in leukemia and malignant lymphoma.

The Medical Service is also engaged in collaborative projects with Urology in the evaluation of bilateral adrenalectomy for disseminated cancer and with Gynecology in the clinical trial of triethylene melamine in ovarian carcinoma.

During the past year, Dr. Irving London has continued studies in porphyrin, cholesterol, and protein metabolism. The metabolism of hemoglobin and bile pigment in polycythemia vera has been studied and has revealed a normal erythrocyte life span of about 120 days. Investigation of the enzymatic mechanisms involved in the synthesis of heme is being pursued with the aid of non-intact erythrocyte systems in vitro. This work, in conjunction with Dr. Elhanan Dimant and Dr. Nicholas B. Myant, is concerned with the effects of agents such as folic acid, vitamin B_{12}, various cations, and lead on the synthesis of heme. The metabolism of cholesterol and phospholipids in a patient with xanthomatosis and hypercholesterolemia has been investigated in conjunction with Dr. Haiyim Schwarz. The results of this study lend further support to the view that some patients with hypercholesterolemia have a relatively diminished capacity for the disposal of serum cholesterol. In conjunction with Dr. Helen M. Ranney, studies on the capacity of various tissues to form antibody in vitro are being continued. These studies have demonstrated antibody formation in spleen, lung, bone marrow and liver, and further investigation of the mechanism and sites of antibody formation is in progress.

Department of Neurological Surgery

Malignant brain tumors are not rare. Because of the difficulty of early diagnosis of these tumors and because normal brain tissue cannot be sacrificed in their radical removal, surgical therapy offers relatively little value. Dr. J. Lawrence Pool, Professor of Neurosurgery and Chief of the Neurosurgical Service at the Neurological Institute, has been in the forefront in the investigation of other therapeutic approaches to the important problem of brain tumors.

Dr. Pool and his associates have treated 25 patients with glioblastoma multiforme with the guanine analog, 8-azaguanine. This drug has proved active against these tumor cells in vitro (see Hirschberg, Murray). In the clinical trials it is felt that some of the patients have benefited from 8-azaguanine treatment as they are still surviving beyond the expected period. In other patients with this tumor there appears to be no effect. Further trial of 8-azaguanine and allied compounds is being continued using different routes of administration and larger doses.

Dr. Ernest H. Wood, together with Dr. Juan M. Taveras and Dr. J. L. Pool, has demonstrated that glioblastoma multiforme can spread to the spinal canal where it forms tumor nodules. In evaluation of such patients with repeated myelograms, these investigators demonstrated that radiotherapy could produce a reduction in the tumor.

Dr. J. L. Pool and Dr. Abbie Knowlton have studied the effect of hypophysectomy in a patient with a functional, malignant adrenal carcinoma. Although the pituitary ablation failed to modify the course of the tumor, valuable information was obtained on the effects of the procedure on endocrine and electrolyte balance. This may prove to be very important in the further investigation of hypophysectomy for the control of malignant tumor growth.

Dr. James B. Campbell has been engaged in an extensive research program on techniques of administering radioactive gold seeds to specific parts of the brain. This study is aimed at accurately controlled internal radiation therapy of malignant brain tumors. The work has already shown great promise with regard to precise localization of radiotherapy and the extent and changes in tissues and vessels which follow.

Dr. Thomas Bridges has continued his important clinical research program on the neurosurgical control of pain in advanced neoplastic disease. Criteria for the selection of patients and for the appropriate operative procedure have been better established. This has become a significant factor in the total management of cancer in late stages of disease.

Department of Obstetrics and Gynecology

The cancer research in the Department of Obstetrics and Gynecology, under the overall direction of Dr. Howard Taylor, is carried out on the gynecologic wards of The Presbyterian Hospital and in the Francis Delafield Hospital and in a number of laboratories. These laboratories include the general laboratories of the Department on the 16th floor of the Medical School, section for steroid chemistry on the 15th floor of the Cancer Research Institute and a series of small rooms in The Francis Delafield Hospital.

In describing the work, it is useful to note that it falls under three principal headings. The biochemistry of the steroids and the clinical application of methods for their measurement form one large area. Investigations depending on morphological studies, correlated at least in part with the biochemical qualities of neoplastic tissue, form a second area of work. Finally a number of clinical studies are of course in progress.

Steroid Hormone Research

Technical Procedures

The laboratory of Dr. Seymour Lieberman, with his associates, has been concerned with the development of new techniques for the separation, isolation and identification of certain steroids. Certain particular fields of research should be noted.

A technique for efficient chromatographic analysis has been developed, which makes it possible to resolve and determine quantitatively the various ketosteroids present in urine samples. The individual components thus isolated can be identified by infrared spectroscopy, thus permitting a qualitative as well as quantitative analysis. The chromatographic analysis employed involves a technique known as "gradient elution" wherein the concentration of the eluent is gradually and continuously increased throughout the chromatogram. A communication has recently been submitted for publication which described a general mathematical expression which extends and simplifies the existant procedures. With these improvements it is pos-

sible to shorten the time of the chromatogram, decrease the volume of solvent necessary and moreover improve the resolution of the various components. With this technique it has been possible to identify in various urinary extracts the following ketosteroids: 1-androsten-6β-ol-17-one, pregnan-3α-ol-20-one, dehydroisoandrosterone, isoandrosterone, androsterone, Δ^9-androsten-3α-ol-17-one, etiocholanolone, 11-keto-pregnan-3α-ol-20-one, 11-keto-etiocholanolone, 11-hydroxy-androsterone, 11-hydroxy-etiocholanolone and 17-hydroxy-pregnan-3α-ol-20-one. The work on a gradient elution chromatographic analysis is being done in particular association with Mr. T. K. Lakshmanan.

A study of the synthesis of glucuronosides is being conducted by Dr. Sylvia Teich with the assistance of Mr. Paul Levitan. Success has been achieved in preparing such conjugates of a variety of steroids including the hormones, testosterone and 3-monobenzoate of estradiol-17. The study of the water solubilities, biological activities and methods of hydrolysis of these compounds is being continued.

Investigations into the biosynthesis of steroid hormones by means of the perfusion of isolated glands with C^{14}-labelled materials have been begun with Doctors Teich and Cohen and the surgical assistance of Dr. Harold Tovell. For the practical development of these experiments considerable time has been spent on the methods for the recovery of the steroids from the blood, involving comparisons of the extractions obtained by various organic solvents, dialysis and adsorption on charcoal and subsequent elution with organic solvents.

Investigations are also underway in Dr. Lieberman's laboratory on the methods of synthesis and the properties of steroid imidazoles, compounds of perhaps considerable importance.

Effects of Progesterone in the Treatment of Cancer of the Cervix

A clinical study of the metabolism of progesterone and its possible therapeutic effects when administered to patients with gynecologic cancer has also been carried on during the last year. A number of patients, all suffering from cancer of the cervix, were given large doses of progesterone either intramuscularly or intra-

venously. With intramuscular administration, treatment with doses of 250 mgs. daily was continued for a period of about two months. Intravenous administration of progesterone dissolved in human serum albumin solution permitted the rapid increase of the blood levels of the active hormones to high levels so that the length of treatment in cases so managed was only ten days. In spite of reports from outside sources of considerable success in the treatment of cancer of the cervix with progesterone, such clearcut results were not obtained in this series treated here. In a few instances, evidence of slight improvement was present although equivocal. There remains for final study the pathological material from a series of biopsies taken from these patients before, during and after their courses of treatment and also the collected and partly analyzed urine specimens, which will be used to determine the urinary metabolites of progesterone when administered in such large quantities.

Investigation of Steroids Excreted by Adrenalectomized Patients

A clinical study of the nature and amounts of 17-ketosteroids being excreted by gonadectomized and adrenalectomized patients is also underway in association with Dr. William Cohen, Mr. Bert Mond, Mr. George Broussalian and Mrs. Sally Marshall. The demonstration of any residual steroid formation after gonadectomy and adrenalectomy would of course in itself be important. This study is also of value in determining the metabolites following the administration of cortisone and other steroids to such patients without gonads or adrenals.

Adrenal Virilism from Hyperplasia or Adrenal Tumor

The work of Dr. Joseph Jailer and his associates on this subject has been an extension of the investigation begun during previous years with a beginning of new problems.

It has been fairly well established now that the administration of cortisone or Compound F to patients with adrenal hyperplasia and virilism results in a fall of the urinary 17-ketosteroids. On the other hand if an adrenal tumor is the cause of the disease, no such fall occurs. A sufficient number of patients have been studied now

so that it is a fairly reliable test for the differentiation of the two causes of adrenal overactivity.

Further investigations on patients with adrenal hyperplasia together with steroid studies performed on glands obtained at operation, have led to the theory that there is an inborn error of steroidal metabolism in congenital adrenal virilism. It is claimed that there is an enzymatic defect in the adrenal whereby the synthesis of Compound F from one of its precursors, 17-hydroxy-progesterone, is impaired allowing for the secretion of the latter steroid instead of Compound F. The 17-hydroxy-progesterone is in turn degraded, presumably by the liver, to androgenic steroids. These latter steroids are responsible for the masculinization so characteristic of the disease.

The studies on adrenal virilism have led to an interest in the more benign cases of hirsutism with and without menstrual disorders in women. A special clinic has been set up for studying these cases and at the present time about 25 such women are being studied. Efforts are being made on the clinical classification of this condition and the urinary steroids are being fractionated in an attempt to ascertain whether there are any differences from the normal. In addition, certain ovarian and adrenal tests are being performed which will localize the seat of the pathology.

This work is being done in collaboration with Doctors J. J. Gold and Raymond Van der Wiele, a Belgian Government Fellow in Obstetrics and Gynecology, who are working in this laboratory.

Studies on Radiosensitivity

Efforts to establish the relative radiosensitivity of different examples of cervical cancer have continued in the cytological laboratory being conducted by Dr. Saul B. Gusberg. The object of the study is of course a practical one, since it is hoped that it may be possible to determine those types of cervical cancer for which surgical or radiation therapy, respectively, are best suited.

Preliminary studies in Dr. Gusberg's laboratory had indicated that a shift oc-

curs from active to inactive cells in a series of biopsies taken from the cervix during a course of radiation therapy. This shift can be detected by histochemical means and is most evident in those patients who are responding favorably to radium or x-ray therapy.

At the present time a study of the normal cell reactions of the vaginal epithelium has also been undertaken and a histochemical evaluation of the changes in the two nucleic acids of the cells, desoxyribonucleic acid and ribonucleic acid, which apparently accompany these morphological changes has been started. The application of these newer techniques is being started with the aid of advice from Dr. Margaret Long.

Histochemical and Biochemical Studies in Tumor Differentiation

In the discussion of cancer as a problem, it is generally customary to regard this disease as a specific entity, as it occurs in any particular organ, differing in kind both from the normal tissues and benign tumors of the given tissues. It is also possible, however, to regard malignancy as a quality, present to a varying extent in the different individual examples of tumors of some special organ.

Particularly in the adenocarcinomas of the ovary and the endometrium, there appears to be an unbroken series of transitional forms, from the stage of benign tumor or hyperplasia, through types of borderline malignancy, to the fully developed metastasizing cancers. The basic plan of the present research project is to try to determine underlying histochemical, cytological or biochemical characteristics which correspond to this series of forms in which the quality of malignancy is manifested to a varying degree.

The initial phase of this study is the collection of cases of ovarian carcinoma and carcinoma of the endometrium and their histological grading based on multiple sections stained with the classical hematoxylin-eosin techniques. A total of 17 gynecological tumors have so been studied, the majority adenocarcinomas of the ovary. As a first step, multiple sections from various parts of the tumor, varying in number from 5 to 15, were separately studied to determine the consistency of tu-

mor structure in different areas. Although some exceptions were to be noted, the degree of consistency seemed to be very high. Sections were then examined by special tissue reactions for desoxyribonucleic acid, ribonucleic acid, alkaline phosphatase, glycogen and various lipids. Some evidence of a correlation between degree of malignancy and quantity of nucleolar ribonucleic acid has been an initial observation.

As a second phase of this correlated study, larger quantities of the same tumors have been sent to Dr. D. V. N. Reddy in the Delafield Hospital laboratories where biochemical determinations are being made of the concentrations of nucleic acids and their hydrolytic products. Particular attention is being given the study of cell particulates and the finer composition of the two types of nucleic acids in various cell fractions.

Experiments are also in progress to study the enzymes, succinic dehydrogenase, ribonuclease, desoxyribonuclease, d-amino acid oxidase, xanthine oxidase and transaminase. Studies of the B-vitamins (B-1, B-2, and B-6) are also being set up for the purpose of correlating vitamin content and the enzymatic action of these tissues of varying degrees of malignancy. A study of the amino acids in this tumor material is also projected.

As a third phase of this study of ovarian and endometrial cancer of various grades, Dr. Helena de Roetth is examining particular metabolic processes in ovarian tumors of different grades. Chemical determinations of the lactic acid production of all tumors, under identical conditions, is being carried out as a result of the known enhancement of lactic acid production in neoplastic tissue. The activity of certain enzymes such as hexokinase will also be studied since this enzyme is important because of its responsibility for the incorporation of sugars in the glycolytic cycle and because of the observations that in neoplastic tissues the marked increase in the utilization of sugars which might mean an increased activity of this particular enzyme system. The use of C^{14}-labelled intermediates, such as glucose, pyruvate and acetate, will be used to elucidate, if possible, metabolic pathways and relative

oxidation rates in the various grades of tumors. Also planned is an electrophoretic study of the soluble proteins of these ovarian and uterine tumors.

Finally, material from these tumors is being sent for independent study of cellular behavior by Dr. M. J. Kopac and his associates in the Biological Laboratory of New York University College of Medicine. In that laboratory, the tumors will be studied on the basis of single cells, particular attention being given to the cell constituents after they have been layered in the cell by differential centrifugation; the growth characteristics of ovarian cancer cells, in tissue culture, obtained from the ascitic fluid from the peritoneal cavities of patients with advanced ovarian cancer will be studied.

The Transplantation of Human Ovarian Tumors

Dr. Harold Tovell has studied the biological activity of 15 human ovarian tumors with respect to their ability to survive, grow and differentiate in alien species. Special attention has been given to the transplantability of ovarian tumors into the anterior chamber of the guinea pig eye.

Heterologous transplantation studies have been successfully carried out with a number of varieties of malignant tumor and certain theories have been evolved regarding the characteristics of malignant tissue in general. Some of these theories remain to be tested when applied to specific malignancies such as those seen in human ovarian tumors. Dr. Tovell's results obtained to date, based on a small number of cases, suggest that previous concepts are only partially tenable.

Further studies are in progress employing slightly different techniques which promise favorable results. Eventually it is planned to employ heterologously transplanted human ovarian, cervical, and endometrial tumors for cytological and biochemical studies. It will be noted that such techniques for the study of human malignancies have only been recently made available for practical purposes and may open an important new field in gynecology.

Miscellaneous Pathological Studies

A number of clinical-pathological studies have been in progress and a number of

publications have resulted from this type of work in the department during the last year.

Dr. Harold Speert has studied the patterns of endometrial change preceding the development of endometrial carcinoma, by collecting with considerable effort specimens of endometrial biopsy or curettings obtained at some date prior to the appearance of the carcinoma. In order to examine the current hypothesis concerning the relation of estrogen stimulation of cervical cancer, Dr. Speert has also studied the endometrium in 134 cases of cervical cancer. Since the endometrium affords a reliable measure of estrogen activity and since no specific type of endometrium was found in association with carcinoma of the cervix, it is believed that there is little reason to assume an immediate estrogenic factor in the etiology of cervical cancer.

On account of the new clinical problem presented to the gynecologists by the recognition of the so-called intra-epithelial carcinoma of the cervix, Dr. Earl T. Engle has become impressed with the importance of perfecting techniques for the investigation of the basement membrane. Work has been begun toward the development of methods for demonstrating the basement membrane and for the hyaluronic acid-sulphydryl in the ground substance.

Dr. Saul B. Gusberg, with Dr. David Moore, has continued his study of the life history of cervical cancer and has completed a report demonstrating the pathological course of this disease, beginning with intra-epithelial cancer and showing the various methods and stages of dissemination and metastasis.

Dr. Equinn Munnell and Dr. Ferdinand Flick are studying the histology of the endometrium in cases of abnormal uterine bleeding unassociated with obvious gross pathology.

Clinical Studies in the Cancer Field

An interesting technique for the refinement of diagnosis in pelvic cancer is being developed by Dr. Salim J. Dalali in association with Dr. Arnold Bachman. The technique is the use of what has been called the "pelvic venogram", produced by the

injection of a radio-opaque medium into the veins of the lower extremity, to demonstrate the venous system of the pelvis. The method is of use, it is believed, in differentiating edema of the lower extremities due to lymphatic obstruction from that due to venous obstruction and for indicating the point of bifurcation of the common iliac veins, "Point C", the area where involved pelvic lymph nodes are most frequently found and a point toward which the x-ray beam should be directed in therapy. The method has incidentally demonstrated the communication between the pelvic venous system and the lumbar vertebral system of veins.

Clinical reports have been published by Dr. J. Corscaden, Dr. S. Gusberg and his associates on the use of radium needles in treatment of cancer of the cervix and on the general subject of treatment of cancer of the endometrium.

A rather special area of study has been the investigation by Dr. Byron Butler of the possible use of hypnotism for the control of pain in cases of advanced cancer. This work has been completed and has led to the acceptance of a paper for publication.

A general plan for the combined radiation and surgical treatment of cancer of the female reproductive tract in the Presbyterian Hopsital and in the Francis Delafield Hospital continues. This plan was arrived at in November of 1951 as a result of a series of conferences between the radiotherapists and gynecologists of the two hospitals. The plan has been completely reviewed by a similar group during the last few weeks and the revised formulation will be put into effect to give consistency to the theory of treatment of the two institutions. The plan to date appears to have worked well and it is believed will yield information which could not be obtained if random decisions were made for each new individual case.

Department of Pathology

Studies on cancer have been carried out by the Department of Pathology, under the auspices of the Institute of Cancer Research, both at the Francis Delafield Hospital and in the Medical School building itself. The latter program has been carried out by Dr. Theodore F. Zucker and Dr. Lois M. Zucker of the Division of Clinical Pathology. They have continued their studies on the "toxicity" of acetylaminofluorene (AAF), one of the compounds commonly employed to produce cancer experimentally in animals. These toxic effects include, among other things, an inhibition of body growth. If the toxic effects of this compound can be minimized, it will be possible to study cancer production in animals which are otherwise growing at a normal rate. As reported already, these workers have found that certain natural foodstuffs contain factors which counteract these toxic effects. They do this without destroying the ability of the compound (AAF) to produce cancer. Current work shows that this "counter-toxic" factor is readily inactivated whenever efforts are made to isolate it from natural sources. Nevertheless, methods have been found which permit a 50-fold increase in the potency of preparations. The time may not be far distant when the compound will be available in sufficiently pure form to permit clinical and metabolic studies to be made.

The program at the Francis Delafield Hospital has been carried out under the direction of Drs. A. P. Stout and Edith Sproul.

Since January 1953 a laboratory for special histochemical studies has been under development by Mr. Martin Blutinger and Dr. Edith Sproul. This is being used both for diagnostic services beyond ordinary techniques as well as for a research program. Trial of new materials and techniques and improvement of apparatus have led to the satisfactory use of methyl green pyronine for differential staining of RNA and DNA. This finds its greatest use in identifying myeloma cells in tissues. A study of bone marrow and lymph nodes is being conducted.

Histochemical demonstration of alkaline and acid phosphatase is now deemed adequate as an aid in differentiation of carcinoma of the prostate from that of other

organs. The acid phosphatase secretion of breast cancers is being investigated.

The histochemical and manometric measurement of succinic dehydrogenase activity is being used by Dr. Edith Sproul, Mr. Martin Blutinger and Dr. Walter Menaker as a check on in vivo inhibition of the enzyme. Progressive experiments are being carried out to determine the maximum degree of chronic inhibition that can be obtained without lethal effect. The tissues are being studied for cytologic alteration and growth pattern. This is the first step in a program of study of the effect of inhibition of various components of respiratory systems on morphologic character, growth and isolated cell constituents of all organs.

An autopsy survey for the incidence of occult carcinoma in the skeleton is being conducted by all the members of the Pathology and Radiology staff. The data obtained by the interpretation of post-mortem x-rays and extensive sectioning of the skeleton is being organized for publication.

A search for occult carcinoma of the skeleton has been made by Dr. George Hyman using the Wright's stained smears of aspirated marrow. This is in collaboration with Dr. Edith Sproul and Dr. Thomas Petrick, who are studying the paraffin embedded aspirate using a variety of stains as well as application of other stains to the smears.

Further work in the hematology laboratory by Dr. George Hyman to date has centered about routine laboratory tests for measurement of hemolysis and supplemented by the Ashby technique of following transfused red cells. At present it seems that hemolysis is an important factor in the anemia associated with cancer. Another program has employed a scintillation counter, the radioactive isotope iron, Fe^{59}, and factor IV-1 (of Cohn), the iron binding globulin. These studies with intravenous iron have yielded many interesting facts, among which one of the more important is probable demonstration of normal red cell production in the anemia which occurs in neoplastic disease.

Orientation experiments have been started on tissue culture of mouse milk breast cancer virus using embryonic mouse tissue. The technique of Enders is being followed by Mrs. Lucille Seguin with Dr. Samuel Graff.

Mr. Robert Butler, working under the guidance of Dr. Edith Sproul, has been growing squamous epithelium and epidermoid carcinomas in tissue culture and studying their ability to convert l-ascorbic acid to d-glucose.

Clearing and dissection of radical resections and areas of the body at autopsy has been continued by the Pathology staff. This has as its purpose the expansion of our knowledge of variations in lymph node pattern and the establishment of a greater degree of accuracy in demonstration of existing metastases. This has been especially useful for observation by Dr. George Montgomery of the Gynecology Department, working under the supervision of the Pathology Department, of the pelvic lymph node metastases which had received prior radiation.

In association with Dr. Austin Kutscher and Dr. John Piro of the Dental Department, the possible toxic effect on the gum of many drugs used as local anesthetics or antibiotics is being studied. The study of the effect of efocaine on the soft tissues has been completed and accepted for publication. Its effect on nerves is being studied by dissection of the mandibular nerves at autopsy on patients who had received the drug. In addition, various means of obtaining material from the gum for histologic study other than by biopsy are being followed. These include gelfoam, needle aspiration, gauze sponging and imprints. The gelfoam "biopsy" study has been completed.

The detection by frozen section of open perineal biopsy and the incidence at autopsy of occult carcinoma of the prostate is being continued by the entire Pathology staff in conjunction with the Department of Urology.

A study of the diffuse changes in the lungs detected at autopsy on patients dying in severe respiratory failure associated with certain types of obstruction of pulmonary lymphatics is being made by Dr. Edith Sproul.

Juvenile fibromatosis is a process which affects infants and young children either as a solitary nodule, as multiple nodules, as a progressive growth or as a generalized process. It has been called by many different names, a fact which has militated against its proper understanding. Using 38 cases at his disposal, Dr. A. P. Stout is studying the process as a whole in an attempt to determine its rela-

tionship, if any, to fibrosarcoma and progressive myositis ossificans and to evaluate different forms of treatment.

The recognition of benign pigmentation of the cornea and conjunctiva and its distinction from malignant melanosis and melanoma is a matter of great importance from the point of view of diagnosis and treatment. Using the large collection of about 120 cases accumulated by Dr. A. B. Reese at the Institute of Ophthalmology of this Medical Center, Dr. Reese and Dr. Stout are attempting to establish reliable criteria for the above purpose.

Pain and limitation of motion in the pelvic and spinal joints has been assumed by Dr. C. M. Gratz to be due to obliteration of adjacent tissue spaces by fibrosis thus limiting joint motion without any ankylosis of the joints themselves. Dr. Stout is attempting to assist Dr. Gratz to demonstrate this point by a study of the extraarticular soft tissues.

Occasionally a tumor-like proliferation of connective tissue occurs, usually but not always as a result of irradiation, in which the cells assume the bizarre anaplastic appearance of malignant sarcomas without being true neoplasms. Very little is known and less understood about this process. A group of such cases is being studied by Drs. Nathan Lane and Stout.

The peculiar tumor of the breast called cystosarcoma phyllides is one which occasions speculation about the form of treatment proper to be used because reliable criteria enabling one to distinguish between its benign and malignant forms do not exist. The available cases are being studied by Drs. Jane Lester and Stout in an attempt to elucidate this matter.

Benign and malignant tumors of the salivary and mucous and serous glands have been the object of intensive study by a great many groups but with little progress until recently. The material at this Medical Center has been intensively studied by Drs. David Stump and Stout with the special objects of learning how many mixed tumors become malignant, the biological course of the different types of salivary gland cancers and the course and behavior of lymphoid infiltrations of the salivary glands.

In addition to the reticulum cell lymphosarcomas, it has become apparent that reticulum cell sarcomas can develop in the soft tissues away from normal lymphoid stations. Presumably these develop from histiocytes. Their existence has been heretofore recognized in bone marrow but not in the soft tissues. A group of such cases is being studied by Drs. R. Lattes, R. R. Milch and Stout.

A rare and extraordinary lesion occurs which causes gradual disappearance of one or more bones. Apparently this is due to a peculiar form either of hemangiomatosis or lymphangiomatosis. A global study of this condition is being conducted by Dr. L. W. Gorham of the Public Health Research Institute of the City of New York, Professor Wright of Albany Medical School, and Dr. Stout.

Although malignant melanoma has been the subject of innumerable studies, the exact degree of malignancy of its various morphological types is still a matter of conjecture. In collaboration with Dr. R. Lattes this feature is being studied by Dr. Stout in the hope of obtaining a more reliable guide to lymph node therapy.

In collaboration with Drs. B. S. and E. T. Oppenheimer, Dr. Stout is continuing the investigation of the various types of plastic films which will induce sarcomas in certain rodents. An attempt to find the nature of the carcinogenic agent in the various plastics is now in progress.

Dr. C. D. Haagensen and Dr. Stout are continuing their studies of various breast lesions, in particular they are concerned at present with the papillary carcinomas of the breast and their possible relationship with benign intraductal papillomas.

Cured cases of reticulum cell sarcoma of the spleen are exceedingly rare. A report of a ten-year survival is being prepared for publication by Dr. John Powers of the Mary Imogene Bassett Hospital, Cooperstown, N.Y., and Dr. Stout.

True adenomas of the oral cavity are very rare. A study of the nature and occurrence of such growths has been undertaken by Drs. M. Heinemann and Stout.

Department of Pediatrics

During the year 1952-53 the Department of Pediatrics, under the direction of Dr. James A. Wolff, has continued its investigations of chemotherapeutic agents in childhood leukemia.

From July, 1952 to the end of March, 1953, investigations have been limited by a scarcity of clinical material. During this period 6 patients with acute leukemia were treated with anti-folic compounds, particularly amethopterin. In several of these cortisone therapy was also given when response to the anti-folic compound was not or no longer obtained. In several, leucovorin was given to reverse amethopterin toxicity. Although good remissions were obtained in 50 per cent of patients, all these children are now dead.

At the end of March, 1953, 6-mercaptopurine (purinethiol) became available for trial as a chemotherapeutic agent in acute childhood leukemia. Since that time clinical material has been more plentiful. To date 11 children with acute leukemia have been treated with 6-mercaptopurine. The results have been encouraging and appear to equal those with any of the other agents which have been employed in the past. Six patients have had clinical and hematologic remission. In 2 others clinical remissions have occurred. One patient suffered severe bone marrow depression, necessitating discontinuance of the drug. One patient was treated in a terminal state, and one patient has had insufficient follow-up for evaluation. All but one of these patients are now alive.

Because of the encouraging results with 6-mercaptopurine, it is planned to continue these studies further.

Department of Radiology

A. Presbyterian Hospital

Portal venographic studies are being made by Dr. Alfred Berne, through injection into the spleen to demonstrate metastatic neoplasm of the liver, retroperitoneal disease and to study portal hypertension.

Dr. Samuel Madell is making a study of cases with total colectomies and ileosigmoidostomies as regards compensatory dilatation of the terminal ileum following colectomy.

Dr. Jerome Nadelhaft continues his work on the evaluation of the effect of radiotherapy on salivary gland tumors in a collaborative study with Dr. Arthur Purdy Stout.

A study of the practical adaptation of graphics in localizing radium implants is being carried out by Dr. Morton Kligerman and Prof. Howard W. Vreeland, School of Engineering, Columbia University.

The use of Dramamine and Pyridoxine in radiation sickness in a clinically controlled experiment has been initiated by Dr. M. Kligerman, Dr. Anna Silverman and Dr. John Fertig, Professor of Biostatics of the School of Public Health.

Dr. Juan Taveras and Dr. Gerhart Schwarz are working on a new type of stylet which would enable them to aspirate pantopaque after myelography with greater ease.

Dr. Catherine Dalton and Dr. Solomon Schwartz are studying the anatomy of the paravertebral shadow in the thoracic region and the causes of variations therein. They have concluded that these variations are caused by posterior pleural reflections.

Dr. Virginia K. Frantz of Surgical Pathology and Dr. M. Kligerman have undertaken a study of carcinogenesis in the thyroid of the Long-Evans rat from internal (radioactive iodine) and external radiation.

Dr. Gerhart Schwarz has been working on the design for a slide rule for the correction of anatomical measurements in roentgen cephalopelvimetry. This is at present being manufactured and will be distributed by Picker X-ray Corp. in the fall.

Dr. Ross Golden, in collaboration with Dr. Pablo L. Morales, has made a study of 199 cases of organic disease of the small intestine with particular attention to the various malignant diseases to determine the efficacy of x-ray examination in the diagnosis of these diseases. It was found that in approximately 90 per cent of the cases examined by x-ray methods, an abnormality was demonstrated.

Dr. Kent Ellis is studying the pattern of mucosal folds on resected stomach specimens, comparing them with the folds as demonstrated on x-ray examination of the stomach done pre-operatively. The objective is to study the effect of carcinomas and of inflammatory disease on the mucous membrane as it concerns the problem of differential diagnosis.

B. Francis Delafield Hospital

I. Radiotherapy

Report of clinical activities - A total of 843 patients were treated by roentgen therapy from July 1, 1952 to June 15, 1953. The number of patients treated with 2 million volt x-rays was 494, and 349 were treated with 250 KV or lower voltages. This represents an increase of 291 patients over the 552 patients treated during a similar period in the preceding year. Besides the 843 patients treated by x-rays 22 were treated by radium. The cancer in the majority of the patients referred for radiotherapy is so extensive that only palliative radiation doses seem justified in most.

In addition to the treatment with x-rays 415 patients were examined for follow-up or were seen in consultation.

Joint new patient and follow-up clinics have been continued with the Departments of Gynecology and Surgery. The treatment of cancer of the bladder with 2 million volt therapy has been continued as a research project with the Genitourinary Service.

Report of experimental work - Radiation of liver metastasis, as a clinical research problem, has been continued by Dr. Ruth Guttmann in collaboration with members of the Medical Department.

Standardization of radiation doses administered to pelvic lymph nodes in gyne-

cological cancer has been attempted. Some of this work has been done in collaboration with venographic studies by Dr. S. Dalali of the resident staff of the Gynecological Department.

Dr. Emanuel Tulsky, Dr. Guttmann and Dr. M. Lenz have continued studies of the effects of 2 million volt x-ray therapy on carcinoma of the lung.

Dr. Tulsky has made special studies on the x-ray dosage applied to the internal mammary lymph nodes with 250 KV and 2 million volt x-ray therapy.

Dr. Anna Goldfeder has continued her studies on the minimal lethal x-ray tumor dose required by various autogenous mammary adenocarcinomas. She has also continued to study the time factor in fractionated radiotherapy.

Drs.R. Deterling and E. Tulsky have started an investigation on the possibilities of sterilizing, by x-ray therapy, segments of aortic tissue removed post-mortem. These aortae are to be used as homografts.

II. Diagnostic Radiology

Under the direction of Dr. Arnold Bachman the extension of angiography and venography for the localization of tumors in various parts of the body has been developed. Particularly revealing have been the bilateral transcutaneous femoral vein venograms carried out in collaboration with the Gynecology Service. Using this technique the normal pelvic vein pattern and the changes in patients with pelvic tumors have been studied. It has been intensely interesting to observe the vascular connections between the pelvis and the venous plexuses of the vertebral column which are demonstrable by this technique.

Tomographic studies of the base of the skull have continued with demonstration of tumor extension not recognizable by conventional x-ray films. The tomographic technique has also proved valuable in studies of the larynx in patients with unilateral cord paralysis.

Routine and tomographic roentgenograms of the dorso-lumbar spine have been made post-mortem on cases immediately prior to necropsy. The spines of these cases have been removed and carefully studied by the Pathology Service. A report of the correlations between x-ray and pathological findings is in preparation.

C. Radiological Research Laboratory

The work of the Radiological Research Laboratory may be considered in three broad categories, basic radiophysics as related to the measurement of radiant energy, basic radiobiology, and clinical applications. In the 1951-52 report, the fundamental considerations underlying the radiophysical and radiobiological studies were outlined; these continue to be governing principles for selection and development of research problems. Of the various subjects under investigation during the past year, those mentioned below have been selected as having more immediate bearing on cancer research.

Physics

Dr. G. Failla and Dr. H. H. Rossi, with a group of research associates, have concentrated on measurement of radiation dosage. For neutron measurements, ionization chambers have been constructed of tissue-equivalent solid, containing tissue-equivalent gas. That is, the entire chamber has the atomic constitution of average soft tissue. Such chambers are not necessary for x-rays, but they are essential for neutrons. An extension of this work, carried out in collaboration with Dr. E. Stickley, has been the measurement of tissue dose in brain tumors which have been impregnated with boron and irradiated with slow neutrons in the nuclear pile at Brookhaven.

Determination of dosage from radioactive isotopes distributed in tissues becomes increasingly important. First approaches to these problems assume homogeneous distribution throughout masses of tissue, but actually this situation rarely if ever exists. Not only is distribution varied from one organ to another, but from cell to cell, and even in different parts of a single cell. Dr. Failla and Miss Patricia McClement have developed a wall-less ionization chamber directly applicable to a detailed study of dosage in small localized regions, but only a few results are as yet available.

Other problems in basic radiophysics, instrument design and development are also being carried forward.

Biology

Dr. Roberts Rugh and his group have studied histopathological phenomena in acute and delayed radiation death. The effect of irradiation in utero on fertility, organ development, and growth have been investigated both with x-rays and radioactive iodine.

Dr. Ludwig von Sallmann has continued his work with radiation cataracts. With the cooperation of Drs. C. A. Tobias and H. O. Anger, of the Donner Laboratory of Medical Physics of the University of California, studies were extended to high energy deuterons and alpha particles.

Many investigators have tried to find means for protecting against the effects of radiation. Some time ago Jacobson (at the University of Chicago) showed that if the spleen was shielded the effects of whole body irradiation were markedly diminished. It appeared of interest to reverse this procedure, that is, shield the whole body and irradiate only the exteriorized spleen. When this was done by Mr. Bruce Allen, the result was found to be the development of a profound anemia in rabbits whose exteriorized spleens received 1600 r. This suggests many leads in the study of the mechanism of production of radiation anemia, effects of transfusion, effects of other protective agents, etc.

Radioactive Isotopes

The Radicisotope Laboratory, under the supervision of Dr. Edith Quimby, operates as a receiving, standardizing, and dispensing center for all isotopes used clinically in the Presbyterian Hospital. In addition, the laboratory personnel assist in setting up various isotope research programs throughout the institution.

Therapy with radioactive isotopes - Radioactive iodine continues to be used in treatment of some thyroid cancers as described in last year's report. There are no new developments to report in this program under the leadership of Dr. Virginia Frantz, nor in the use of radioactive phosphorus in leukemia and polycythemia, under Dr. Harold Jacox and Dr. M. Kligerman. The use of radioactive colloidal gold in treatment of pleural effusion or ascites due to carcinomatosis, with Dr. M. Kligerman and Dr. David Habif, has afforded some remarkable instances of palliation.

This work was reported at the 1952 meeting of the American Radium Society. No new therapeutic procedures have been developed. (Treatment of hyperthyroidism with radioactive iodine continues to be important, but this is not part of the cancer program.)

Diagnostic and tracer studies with radioactive isotopes - Radioautograph studies for thyroid cancer cases continue to be made as described last year; some new techniques have been developed. Studies of blood volume, sodium space, and cardiac output are applied to cancer and other patients; these were developed mainly in the Department of Surgery. In collaboration with Dr. James Austen, studies have been started on protein turnover in cerebrospinal fluid, the tracer being human serum albumen labelled with radioactive iodine.

Radium and X-ray Physics

Dr. Edith Quimby has investigated problems connected with radiation dosimetry. The work commenced last year on modifications of radium dosage systems has been completed and a paper accepted for publication.

Studies on dosage determinations in rotation therapy have been continued. The method for calculation when the patient, sitting or standing, turns in a horizontal beam, has been greatly simplified. When the recumbent patient rotates in an oblique beam, the problem is much more complicated. Methods of calculation were worked out, and to check these a rotating phantom was constructed and a Baldwin-Farmer electrometer secured, equipped with a number of very small ionization chambers. With these, dosage could be measured at a number of points under conditions simulating actual treatment. Good agreement was obtained between calculated and measured values. It now appears that calculation methods in this type of treatment can also be considerably simplified, but this study is continuing.

As an adjunct to the work with this second type of rotation, studies were begun on the use of wedge filters to produce more uniform distribution of radiation in the tissue when oblique beams are used. Much remains to be done on this problem.

For any accurate dosage determinations in x-ray therapy it is necessary to have isodose charts, showing the complete distribution of radiation in the beam as it

passes through tissue. Construction of such charts is based on ionization measurements in phantoms and is a laborious and time-consuming procedure. Methods have been devised in Britain for developing such charts by calculation, when the doses along the axis of the beam have been measured. A project has been commenced to supply the necessary basic data (much of it is already available) to recalculate the tables from which the constants for calculation are drawn, simplify as much as possible the application of the formulae, and make the method available in the United States, where at present it is little appreciated or understood. Considerable work has been done on this, but much more remains to be done.

An important part of determination of dosage in interstitial radium therapy, and perhaps the most difficult, is the correct localization of the radium sources with reference to anatomical points of interest. This is done with the aid of radiographs taken in two fixed positions. Several methods are available, all have limitations and are time-consuming. Dr. Howard Vreeland of the Department of Engineering of Columbia University, has been working on this problem. He has devised a method believed to be simpler and quicker than any others currently available.

Department of Surgery

Dr. Margaret Murray has directed a number of collaborative projects in which tissue culture techniques of the Laboratory of Cell Physiology have been employed.

In collaboration with Drs. A. P. Stout and R. Lattes, Drs. Thelma Flint and Murray have continued the long-standing work of the laboratory in culturing rare or disputed human neoplasms, accumulating information on their form and behavior in vitro, as an aid in classification and diagnosis.

With Dr. Erich Hirschberg, Mrs. Edith Peterson and Dr. Flint have prosecuted chemotherapeutic studies of human glioblastomas and of the Brown-Pearce rabbit carcinoma. The Brown-Pearce tumor, which in common with the human glioblastoma lacks azaguanine deaminase, has been shown, like the human tumor, to be severely damaged in vitro by 8-azaguanine. A structurally related compound, benzimidazole, is much less effective; the unrelated aliphatic compound 1, 4-dimethanesulfonyloxybutane (Haddow's GT 41) is ineffective on this tissue. On the human glioblastoma, benzimidazole is also less effective than 8-azaguanine; and two other newly synthesized related compounds, benzotriazole and 2-hydroxymethylbenzimidazole, have been found ineffective. Screening of other potentially inhibitory compounds upon the human glioblastoma is under way.

In a study with Dr. E. Chargaff on the mechanism of action of mitotic poisons, Mrs. Helena Benitez has underlined the remarkably specific effect of meso-inositol in reversing the action of colchicine. It is now demonstrated that neither the dextro-isomer, nor muco-inositol nor lipositol will produce this inhibitory effect. She has also shown that a simple heptane ring-compound, tropolone, favors the formation of the spindle and is a more potent antagonist of colchicine than meso-inositol. This latter finding is of especial interest because of the structural-analog relationship between colchicine and tropolone.

Dr. Virginia Kneeland Frantz has continued the study of thyroid cancer, with more detailed study of specimens from radical dissections, in an effort to document routes of spread of the disease, and with more numerous sections to establish possible histological variations in primary tumors and their metastases. These obser-

vations have been supplemented by I^{131} studies - uptake, radio-autographic and screening, - in collaboration with the clinicians and members of the Department of Radiological Research. The Friday noon conferences on Thyroid Cancer have been held regularly with the assistance of the surgical resident assigned to Surgical Pathology. Experimental work is in progress in collaboration with Dr. Morton M. Kligerman and Dr. Edith Quimby. This is a study of carcinogenesis in the thyroid of the Long-Evans rat from internal and external radiation.

Critical evaluation of follow-up data on benign and malignant tumors of the pancreas, both exocrine and endocrine, is in progress and will shortly be published.

In the laboratories of Surgical Pathology, Dr. John Pickren has continued his research on the incidence and topographic distribution of lymph node metastases from carcinoma of the breast. This study is carried out by the method of clearing tissues from block dissections with serial sections of nodes in selected instances. The information thus far obtained from 200 cases has added valuable insight into the spread of cancer of the breast.

Dr. Robert Grinnell has continued his research on the distribution of metastases from carcinoma of the rectum and colon and on the intramural downward spread of carcinoma of the rectum. These studies have provided significant information to the plan of radical surgery of cancer of the bowel.

Dr. David Stump and Dr. A. P. Stout have almost completed a clinico-pathological study of 700 neoplasms of the salivary gland. These tumors have wide variation in their natural history, morphology and response to therapy.

Dr. Gabriel Godman has organized a Laboratory of Histochemical Research which will be widely used in cooperative interdepartmental research. Since its establishment work has been initiated on the histochemistry of regenerating striated muscle and on spectromicrophotometric analyses of nucleic acids in individual cells.

Dr. Cushman D. Haagensen, together with Drs. Michael Heidelberger, Elliott Middleton, Daniel Moore, Leon Dmochowski and Samuel Graff have continued the basic research program on mouse mammary carcinoma. The animal conony which provides the

material for the research includes about 13,700 mice of the Paris R III, Leeds and C57 strains.

During the past year a total of 5,230 litters, including 28,020 mice, have been raised in the colony. A total of 5,104 cc. of milk has been obtained from a total of 3,782 lactating female mice.

As in the past, the environmental conditions of the colony have been kept constant.

The bulk of Paris R III milk has been used by Dr. Samuel Graff to prepare antigens for immunological studies by Dr. Michael Heidelberger. These preparations were of two types, unaltered material prepared by ion-exchange, chymotrypsin, hydrolysis, and differential centrifugation, and a formalinized antigen prepared by contacting the above product with dilute formalin solutions over a period of time. The formalin preparations were employed in the mouse immunization studies which were carried on by Drs. Heidelberger and Middleton.

Quantitative precipitin curves have been plotted for the reaction between purified mouse milk factor (F), furnished by Dr. Graff, and antibodies produced in rabbits. Two different pools of antisera were used. The one pool so tested gave closely similar curves with preparations of F made by two different methods. The course of the reactions is similar to those of other antigens of high molecular weight, since the specific precipitate contains more antigen than antibody over much of the range of the reaction. With the larger amounts of F, supernates from the specific precipitates give reactions with both F and antibody, indicating either degradation or inhomogeneity of F.

One use for curves of total nitrogen precipitated is to permit an assay of mouse milk for its content of F. Two such runs with decalcified (clarified) Paris strain milk indicated a content of the order of 40 μg F nitrogen per ml., or nearly 0.3 mg. F protein per ml: This is probably not a valid result, and the analysis must be repeated after the curve is obtained for sera absorbed with milk protein.

The curves are also used for estimating the purity, on a comparative basis with

F of the present degree of purity, of F isolated from different sources and by different methods. For instance, various samples of F prepared electrophoretically by Dr. Dan H. Moore from mouse breast tumor tissue showed an estimated F content of 19 to 42 per cent.

The mouse milk factor is known to stimulate the production of neutralizing, precipitating, and complement-fixing antibodies in rabbits. The limited cross-reactivity of antibodies to the milk factor with other proteins occurring in the milk of mice of a strain which does not carry the factor, as well as with tissue extracts from factor-free mice, thus far indicates that the milk factor may possess an immunological specificity not present in normal mouse tissues. However, only negative attempts to demonstrate antibodies to F in mice have been reported. Efforts to obtain antibodies in mice were undertaken with formalinized milk factor suspended in Freund adjuvants since this procedure is known to enhance antibody formation.

It was concluded that injection of formalinized milk factor in adjuvants into Paris mice did not stimulate formation of measurable quantities of precipitable antibody. The results in C57 mice were more hopeful, but due to some uncertainties the work is being repeated.

Following the direct analysis for precipitable antibody each serum was examined for non-precipitable antibody. In these tests it was uniformly found that the presence of Paris mouse serum reduced the total amount of precipitable N to approximately 50 to 75 per cent of that shown by control tests without mouse serum. In attempts to explain this effect it was found that normal mouse serum of both strains partially inhibited not only the specific precipitation of milk factor by rabbit antibody, but also the reaction of rabbit antibody to the polysaccharide of Type III pneumococcus with the corresponding antigen, a totally unrelated system. No inhibition was noted, however, when normal rabbit serum was added instead of mouse serum. Electrolyte effects were shown to play no part in the inhibition by changing the amount of saline in which the reaction was run. In the case of the milk factor-antibody system there was the possibility that a cross-reaction serum protein was causing the inhibi-

tion. It was indeed found that mouse serum proteins react to a significant degree with rabbit antibody to the milk factor.

Paris mouse serum has been separated electrophoretically into two crude fractions consisting of beta plus gamma globulins and albumin plus alpha globulins. Thus far it has been found that the beta plus gamma globulins react with the rabbit antiserum to milk factor. In addition, the normal cross-reactivity of this antiserum with Fraction B of mouse milk, i.e., the whey, is abolished by prior absorption of the antiserum with mouse serum proteins.

During the past several years this research group at Columbia University has utilized the facilities of the mouse colony for the production of mouse milk in sufficient quantity so as to provide, on one hand, material for Dr. Heidelberger's immunological studies on the factor, and on the other hand, for isolation and characterization of the factor. It has now been agreed that it is necessary to limit the large-scale production of milk and to use the facilities of the mouse colony in such a way as to obtain more information concerning the presence of the milk factor in various types of preparations of milk and tumor tissue by carrying out comparative studies of the tumor-inducing activity of adequate dilutions. It is hoped that these studies will give information as to the best method of obtaining the factor in the greatest quantity and purity.

Dr. Edward L. Howes has studied various methods of administering 8-azaguanine to experimental animals. Inhibition of sensitive epithelial tumors and epithelization were used as the criteria for dosage. With administration in peanut oil once or twice a day doses of 1/3 or 1/6 as much could be employed. Oral administration of 8-azaguanine required greater amounts for effectiveness (1 1/2 times) and then drug action was gradually lost by some offsetting mechanism that developed in about 3 weeks. However, with very large doses by mouth some degree of effectiveness could be maintained. Mouth administration can be combined with intramuscular injection in peanut oil and smaller amounts of 8-azaguanine may be used. All doses that inhibit epithelial proliferation caused weight loss and loss of appetite. No differential

in dose or means of administration has been found for inhibiting the proliferation of sensitive epithelial tumors in mice versus inhibition of epithelization in rabbits. Both proliferative processes were quantitatively inhibited by the same changes.

Considering that loss of appetite with effective doses limited the intake of proteins and nucleic acids, a low-protein, low-nucleic acid diet was given to mice for one week before 8-azaguanine was started. The control group was kept on the diet alone and the 755 tumors grew at a somewhat slower rate in C57 mice. But when 8-azaguanine was given also in previously ineffective doses (7.5 mg.) I.P. the growth of these tumors was sharply inhibited. This dose alone did not cause loss of appetite.

Antimetabolites of some of the essential amino acids were given in combination with 8-azaguanine to mice on diets deficient in the essential amino acids. By this method, sensitive epithelial tumors could be held to the original size of the transplant for 20 days or growth already established could be stopped. But in both instances growth was resumed as soon as the regimen was discontinued. The conclusion was therefore reached that inhibition of tumor growth by dietary restriction does not cause the tumor to lose its potential for growth. The method of allowing the tumor to grow again after a period of inhibition can be recommended as a method for testing the effectiveness of cancer chemotherapy with transplantation tumors.

Dr. Daniel M. Shapiro has continued his program testing the feasibility of utilizing quantitative biochemical differences between neoplasm and normal host tissue as a guide for the combination chemotherapy of cancer in collaboration with Dr. Maurice Shils of the School of Public Health and Dr. L. S. Dietrich of the Department of Biochemistry. During the past year the following data have been gathered demonstrating that low metabolite concentrations render malignant cells vulnerable to antagonist therapy:

1. Vitamin B6 has been found in lower concentration in a mammary adenocarcinoma (755) than in the majority of normal tissues of its host (C57 BL mice).

2. A vitamin B6 antagonist, desoxypyridoxine, has carcinostatic activity against this tumor.

3. The relationship between low metabolite concentration in the neoplasm and antagonist therapy is brought forth by combined therapy with testosterone and desoxypyridoxine. Testosterone, which has no carcinostatic activity against the 755 tumor, decreased the vitamin B6 concentration in the tumor. Thus, even subcarcinostatic doses of desoxypyridoxine are found to cause marked tumor inhibition in testosterone-treated mice.

4. Studies of a vitamin-B6-containing enzyme, glutamic-aspartic transaminase, reveal it to be present in lower concentration in the 755 tumor than in most of the host's normal tissues. Furthermore, this enzyme is antagonized by desoxypyridoxine. A study of transaminase inhibition reveals that tissues of low enzymatic activity are markedly more sensitive to the in vivo administration of desoxypyridoxine than tissues of high transaminase activity.

A possible explanation of the action of the riboflavin analogue flavotin, in enhancing the carcinostatic action of 8-azaguanine was obtained with the observation that flavotin antagonizes tumor (755) xanthine oxidase. The blocking of tumor xanthine oxidase indirectly inhibits tumor guanase, thereby preventing the inactivation of the carcinostat, 8-azaguanine.

In vitro studies using various 6-substituted, 2-amino-4-hydroxy-pteridine derivatives demonstrates that the degree and type of guanase inhibition exerted by these compounds is markedly affected by the configuration around the 6 position of the pteridine nucleus.

Department of Surgery at the Francis Delafield Hospital

A laboratory and clinical study directed toward the development of a technique for measuring the efficiency of lymphatic circulation in the extremities has been carried out by Dr. David Ju. Dr. Ju has perfected a lymphatic efficiency test based on the use of radioactive albumin.

An analysis of the cause of death and frequency of distant metastases in patients dying from head and neck cancer is now being carried out by Dr. Ramon Llobet.

A study of lymph node metastases from head and neck cancer by means of careful analysis of cleared specimens of neck dissections is being done by Dr. Carl Fiend.

An investigation of the role of internal mammary lymph nodes in breast cancer spread is in progress. This project has included two portions. The first consists of an analysis of 100 autopsy specimens in which the internal mammary lymph chain was dissected and the nodes subjected to microscopic analysis. Assistance in this work was obtained from Dr. Joseph E. Cairncross, Dr. David Ju, and Dr. George L. O'Hara.

The second part of the internal mammary node project is a clinical study involving the application of biopsies of the internal mammary chain in patients with breast cancer as a method of determining operability. To date over 100 cases have been collected in the clinical series.

The principal clinical investigations undertaken by Dr. Joseph McDonald, Chief of the Surgical Service, during the past two years has been the development of plastic surgery techniques for reconstruction of surgical defects in the head and neck following radical excision of cancer. The greatest volume of new material and most significant advancement in this line has been in the treatment of tumors located in the region of the oral-pharynx and laryngo-pharynx.

Division of Urology, Institute of Cancer Research and Francis Delafield Hospital

The active investigative program on cancer of the male urogenital system is under the direction of Dr. Perry B. Hudson. His associates include the following: Dr. John M. Reiner, Dr. Morris London, Dr. Kenneth Tsuboi, Dr. T. D. Price, Dr. Michael Lombardo, Dr. Casimir T. Wolan and Dr. Meir Podberezec.

Research upon the nucleic acids in normal and cancerous kidney and testis has continued. The experiments, based upon studies on the incorporation rates of various components of nucleic acids, have progressed to a point at which publication of the earlier results has been found feasible. Newer vascular perfusion techniques have permitted prolonged studies of perfused human testis and kidney surgically removed in the operating rooms of the Francis Delafield Hospital. During the past year, several opportunities have arisen to study the normal human kidney when that structure has of necessity been sacrificed in cases of ureteral tumor.

Research upon the synthetic enzyme systems in human prostate glands is in progress. The glutamine amide exchange reaction and the glutamine synthesis of normal, benignly enlarged and cancerous human prostate has been under investigation. The results to date have been indicative of alterations in the rate of synthesis in prostatic cancer, but statistical analyses have not to date proved that the values obtained are actually significant. Recent expansion of the program includes the addition of studies on several other enzymes thought to be important in protein synthesis. In addition, human and rodent prostatic slices have been studied from a point of view of protein synthesis as gauged by the rates of production of acid phosphatase enzymes. An attempt is being made to standardize this procedure to a point at which substances may be tested for their effect upon protein synthesis.

The studies upon the phosphatase enzymes of both erythrocytes and prostatic tissue have continued. A refined method, based upon the Bodansky determination which is commonly used clinically, has been developed and is being utilized for precise testing upon human serum and other sources of acid phosphatase. The efforts originally made to distinguish clearly between those phosphatases produced by the prostate gland itself and those produced by other tissues of the body have culminated in a

clear delineation of the kinetics and substrate specificity of prostate acid phosphatase. Progress has been made in the experiments designed to learn the metabolism, or fate, of prostatic acid phosphatase which has entered the blood stream. While this enzyme does not normally occur as an intact protein in human serum, it has long been known to be present in varying amounts in serum when disseminated prostatic cancer has developed. Through knowledge gained in studying this metabolism, attempts are now being made to elevate the level of serum acid phosphatase activity for clinical testing in those cases in which prostatic cancer is present but the phosphatase value is normal. In this way it is believed that two kinds of gain will accrue to the patient: first, unnecessary radical surgery for cancer which has already spread can be avoided; and second, hormonal control measures can be instituted as soon as a definite diagnosis is established by this technique.

The survey of aging males in New York City designed to elucidate more completely the incidence of adenocarcinoma of the prostate gland in the living male population, has progressed during the past year at an increasing rate, and now includes surgical biopsy upon more than 300 males. During this study a classification for prostatic cancer, recently published, has been formulated.

Study sheets for the future statistical analysis of patients evaluated in this survey have been designed. One of the most significant findings in this survey to date has been the fact that physical examination of the prostate gland is an extremely unreliable method upon which to depend for final diagnosis of prostatic enlargements. Frozen section technique, however, has been refined and developed through experience gained at the Delafield Hospital until it now appears to be an established portion of the regular routine for evaluating all obstructing prostatic lesions. It has also been clearly shown in this survey that the incidence of prostatic cancer among those men over 50 years of age who have some degree of prostatic obstruction is twice as high as in the same age group among individuals who have no detectable prostatism.

Although total prostatectomy is, in most institutions, useful in but five per cent of patients with prostatic cancer, the biopsy method referred to here has permit-

ted wide use of curative operations at the Delafield Hospital. During the past 24 months, for example, more early cancers have been subjected to radical prostatectomy at Delafield than at any other hospital in the world, regardless of size. This surgery has been performed, mainly by the resident urologists, with a mortality rate of slightly over one per cent - the lowest on record.

More than 30 bilateral total adrenalectomy operations have been performed for advanced neoplastic disease. The clinical results, as appraised jointly by the Departments of Urology and of Medicine, are such that it has been advocated that the procedure be abandoned for palliation of advanced disseminated prostatic cancer. Adrenalectomy combined with gonadectomy is still being evaluated in advanced mammary cancer.

The laboratory study upon patients subjected to bilateral total adrenalectomy has been extended to include a number of studies upon the steroid constituents of both blood and urine. One portion of this work has been done in collaboration with the laboratories of the Department of Gynecology. Among the accomplishments during the past year is the attainment of a non-steroidal maintenance program for adrenalectomized patients. In an initial experiment upon individuals maintained without steroid administration, it has been established that the 17-ketosteroid level of the urine during maintenance upon ammoniated glycyrrhizin is zero. This seems to establish the previously debated point concerning whether or not tissues in the body other than the gonads and the adrenal cortex are capable of manufacturing ketosteroids.

The studies on ascorbic acid levels in adrenal-cortical tissue begun last year have been submitted for publication. These experiments have been performed on fresh tissue from the operating rooms and have been evaluated before, during and after the administration of adrenocorticotrophic hormones. The work has been performed in collaboration with Dr. Frederic Agate of the Department of Anatomy.

Fractionation of large quantities of human adrenal blood has been made possible by the development of a dependable technique for cannulation of the central adrenal vein. The steroid analyses on effluent adrenal blood have been done both in the

Urological Section of the Institute's laboratories, and through collaboration with the Worcester Foundation for Experimental Biology. A more nearly complete knowledge of the steroid substances elaborated and added to circulating blood by the adrenals in response to ACTH and other stimuli has been gained through these experiments.

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George A. Hyman

SCIENTIFIC REPORT OF THE INSTITUTE OF CANCER RESEARCH

1953—1954

COLLEGE OF PHYSICIANS AND SURGEONS

COLUMBIA UNIVERSITY, NEW YORK

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Introduction

The activities of the Institute of Cancer Research, during the past year, have been increased in laboratory and clinical research, in undergraduate and postgraduate teaching, and in patient care. In the succeeding pages of this Report, brief summaries of the research of the various Departments are presented. The areas of investigation into the problems of abnormal growth have ranged from a description of the effects of anti-tumor compounds on the biological action and physical chemistry of the Hemophilus influenzae transforming principle, to a critical reevaluation of the criteria for radical surgery in cancer of the female breast.

Reference to the 150 publications which have appeared in the academic year of 1953-54 will demonstrate the frequency with which collaborative studies have been undertaken jointly by representatives of basic scientific disciplines and clinicians. This type of cooperative study characterizes an increasing proportion of the investigative work of all major cancer research groups throughout the world. The interchange of knowledge between the laboratory and the clinic is a significant factor in the year to year improvement in patient care, early diagnosis and cure rates. This essential form of research effort is certainly to be encouraged. At the same time it should be recognized by agencies providing financial support for cancer research that equal or perhaps even greater encouragement should be offered to investigations of fundamental cellular phenomena for which there are no pragmatic applications apparent at the moment. Research within the latter category is also described in the body of the Report.

In addition to the usual teaching schedule on neoplastic diseases, the staff undertook two extra assignments this past year. One consisted of a series of thirty-one Radio Alerts on Cancer which were broadcast to physicians of New York and vicinity on the weekly program, For Doctors Only. These were brief discussions on various clinical aspects of cancer. A far more ambitious undertaking consisted of fifteen one hour color television programs which were broadcast from a studio

created in The Francis Delafield Hospital. This series, sponsored by the American Cancer Society, was under the technical direction of the Columbia Broadcasting System and was broadcast, over a closed circuit, to eight cities on the Eastern seaboard and as far west as Toledo, Ohio. The organization of each program was the responsibility of members of the clinical staff of the Columbia-Presbyterian Medical Center. This exploration of a new teaching medium was an interesting, and also a very time consuming, experiment. Preliminary evaluation by the American Cancer Society indicates that the series was well received by the physicians to whom it was directed. It is anticipated that the color moving pictures which were taken of the programs will be valuable for cancer teaching.

At The Francis Delafield Hospital, the cancer research hospital which is administered by New York City and is professionally staffed by Columbia University, twenty-six hundred and twenty-nine new patients were admitted during the past year. This represents an increase of more than fifteen per cent over the preceding year. The physical facilities together with the accomplishments of its staff are commanding increasing recognition for this three year old hospital.

As the record of the year is reviewed, we can recognize some definitive gains that have been made in meeting the practical problems of neoplastic disease in man. We can also note with pride the contributions which investigators of the Institute have made to the understanding of growth. The work summarized in this Report forms a useful part of the world-wide conspiracy against cancer. The year has been well spent and the next one is entered with confidence in a solid framework on which to build.

Alfred Gellhorn, M.D.

May, 1954

Acknowledgments

The efforts of the men and women of the Institute of Cancer Research during the past year have been made possible in large part by grants and gifts from a number of governmental and private organizations. It is with a feeling of gratitude and a sense of continued dedication that appreciative acknowledgment is made to:

The Albert and Mary Lasker Foundation

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The American Urological Association

The Brook Foundation

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The Gerald H. Smith Fund

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The Jane Coffin Childs Memorial Fund

The Lederle Laboratories

The Leggett and Watters Fund

The Life Insurance Medical Research Fund

The McAndrews and Forbes Corporation

The McMillin Fund

The Marguerite Fisher Fund

The New York City Cancer Committee

The New York Orthopaedic Hospital Research Fund

The Research Corporation

The Rockefeller Foundation

The Ruth Estrin Goldberg Memorial Fund

The Schering Corporation

The Schweckendieck Gift

The Squibb Institute

The United States Atomic Energy Commission

The United States Navy

The United States Public Health Service

The Upjohn Company

Department of Biochemistry

The investigations carried out in the past year again concerned themselves with a wide variety of problems, from the elucidation of fundamental cell reactions to the mechanism of action of growth inhibitors.

Dr. Erwin Chargaff and his group have continued their researches in the field of nucleoproteins, lipoproteins, and related subjects. The composition of mammalian deoxyribonucleic acid (DNA), which may be presumed to be an important component of genetic material, has been further studied in detail. The structure of calf thymus DNA has been postulated to be a chain in which tracts consisting principally of pyrimidine nucleotides are followed by stretches in which purine nucleotides predominate. DNAs were isolated from tissues of four mammalian genera; their composition in terms of the nitrogenous constituents varied from genus to genus, but was constant among the various tissues of one genus. Human normal and carcinomatous liver yielded DNAs of identical purine and pyrimidine composition.

An enzyme preparation catalyzing the phosphorylation of nucleosides by phosphate transfer from nucleotides or other donors, e.g. phenylphosphate, was found in malt and in human prostate. This transphosphorylation system seems to be the first instance of a general enzymatic reaction leading to the formation of nucleotides from nucleosides.

In collaboration with Dr. Margaret R. Murray of the Department of Surgery, the antagonism between colchicine in tissue cultures of rat fibroblasts and the simplest structural analogue of colchicine, tropolone, has been studied. Tropolone reverses the metaphase arrest induced by colchicine in a manner similar to meso-inositol, the only previously known colchicine antagonist.

Dr. Zacharias Dische, in collaboration with Dr. Martin Jacubeit, has continued investigations on the mucopolysaccharides of serum proteins. Two new methods have been elaborated, one of which makes it possible to determine the composition of the total mucopolysaccharide of a protein without any preceding

purification; the other permits the evaluation of certain structural properties of the polysaccharide. These methods have been applied to the characterization of the three main globulin fractions of the serum and have disclosed characteristic differences in the composition and certain chemical properties of the polysaccharide of these fractions. Progress has also been made in the purification of the mucopolysaccharides of the serum.

A series of characteristic color reactions of heptose has been described. This method has been applied to the tentative identification and quantitative determination of some of these carbohydrates.

Under the leadership of Dr. Samuel Graff, the Biochemistry Laboratory of The Francis Delafield Hospital, a division of the Department of Biochemistry, is engaged in a number of diversified activities involving the cause and therapy of cancer.

Since it has been established that a number of animal tumors are of viral origin, the hypothesis has been put forth that viruses are responsible for many, if not all, forms of human cancer. Accordingly, Mrs. Ada Graff, Mr. Kenneth McCarty, and Dr. Samuel Graff continue studies on the properties and biological behavior of the mouse mammary carcinoma virus which might have an analogue in the human. Considerable effort has been expended by Drs. L. C. Sze and Graff in efforts to propagate the mouse virus in isolated tissue cultures. This technique, once established, should be applicable to the human disease.

Intermediary metabolism in cancer tissues in contrast to that of normal tissues is under scrutiny in a variety of experimental systems. Drs. Margaret Marshall, Donald Tschudy, Aaron Freedman, Morris Engelman, Mr. Mark Peters, and Mrs. Graff are administering labelled amino acids to patients in an effort to uncover possible faults in amino acid metabolism which might account for the inanition and cachexia of advanced cancer. Mr. McCarty is studying amino acid metabolism in isolated cell cultures. Mr. Alvin Ostashever is perfusing isolated organs with labelled substances. Dr. Sze is studying the role of cellular particulates in metabolic events, with special reference to nucleic acids in embry-

onic development.

Drs. Engelman and Horace B. Gillespie have made good progress in their syntheses of substituted benzimidazoles, benzotriazoles, and quinoxalines for biological applications. With Mr. Sheldon Greer of the Department of Zoology, they have demonstrated microbiological inhibition and mutation with several of the compounds. With Dr. Kathe Liedke, they have shown that a number of the compounds are inhibitors of amphibian development, arresting early cell division. Two of the compounds are of especial interest, 4-methoxy-6-nitro-benzimidazole, and 5-methoxy-7-nitroquinoxaline, which inhibit later differentiating stages without pronounced effect on early division. Dr. Lester Barth, Department of Zoology, is utilizing these compounds successfully in his elegant studies on organizer orientation.

Fractionation and testing of Storrs-Little strain milk known to contain an inhibitor of leukemia in the mouse has gone forward well in the hands of Mrs. F. J. Agate and Mr. Gerard Zagal. Definitive conclusions are anticipated in the next year on the characterization of the active agent. Orienting experiments by Drs. Alvan Barach and Hylan Bickerman, Miss Sylvia Itkin of the Department of Medicine, Mr. Zagal and Dr. Graff indicate a possible therapeutic approach to certain forms of cancer in animals by decreasing of oxygen tension. Comprehensive experiments to confirm, extend, and explain these findings are in progress.

In the absence of Dr. David Rittenberg on sabbatical leave, the members of his group have continued their investigations of the mechanism of enzyme action. On the basis of a study of hydrogen exchange during transamination, a mechanism for the transaminase reaction has been proposed. The mechanism of esterase activity and the exchange of oxygen between organic acids and anhydrides have been investigated with the aid of O^{18}. A method for the determination of the O^{18} content of carboxyl groups has been developed.

During the past year, Dr. David Shemin has proposed a cyclic series of reactions (succinate-glycine cycle) which explains the metabolic behavior of glycine

in its utilization for porphyrin, purine, serine, and methyl group synthesis. The condensation of "active succinate" and glycine produces alpha-amino-beta-adipic acid, which on decarboxylation yields delta-aminolevulinic acid. To fit the concept, the delta carbon of this new intermediate must have the same metabolic functions as the alpha carbon of glycine. It has been shown that the delta carbon of delta-aminolevulinic acid is utilized for the carbon atoms of porphyrins and purines which arise from the alpha carbon of glycine; the new intermediate is the source of all the carbon and nitrogen atoms of heme. It can also be converted to alpha-ketoglutarate, the alpha carboxyl group of which was originally the alpha carbon of glycine. The role of the new intermediate in serine and methyl group synthesis is under investigation.

Dr. David B. Sprinson and his associates have continued their studies on the mechanism of synthesis of the methyl groups of choline and thymine from the beta carbon of serine. In neither process does oxidation to a formyl group occur. This suggests that folic acid, which is implicated in these reactions, can act as a carrier of a hydroxymethyl group or a closely related one-carbon intermediate. In a study of the biosynthesis of sphingosine in nervous tissue, Dr. Sprinson has shown that carbons 3 and 2 and the nitrogen of serine are specifically utilized for carbon atoms 1 and 2 and nitrogen of the base, the remainder of the molecule being derived from acetate. He has found that ethanolamine and ribose are converted efficiently to acetate in the rat, probably by way of glycoaldehyde. Continued investigations of the synthesis of aromatic compounds by bacteria have shown that in the formation of tyrosine the carboxyl group of shikimic acid is replaced by the alanine side chain.

Dr. Stephen Zamenhof has continued, in collaboration with Dr. Hattie E. Alexander of the Department of Pediatrics, his studies on the type-specific polyribophosphate and the transforming principle of Hemophilus influenzae. The polyribophosphate of H. influenzae type b appears to be a chain like that found in pentose nucleic acids, in which the place of the purines and pyrimidines is oc-

cupied by a second similar chain, linked to the first in 1:1'-glycosidic linkages.

The influence of carcinogenic and carcinostatic agents and of other physical and chemical agents on DNA with transforming activity has been further studied. Human and ox DNA with and without H. influenzae transforming principle were tested for their resistance to heat, ionic strength, dehydration, deoxyribonuclease, formaldehyde, peroxide, ferrous iron, and other mutagenic and protein denaturing agents. The loss of stability to heat in specified conditions was found to be a sensitive criterion of injuries to the DNA molecule. A series of nitrogen mustards was tested for their inactivating power for transforming principle; the order of effect on DNA seems to be the same as the order of carcinostatic activity of these compounds. The DNA which survives the mustard treatment becomes unstable to heat or storage though it retains its biological activity. Of other carcinostatic agents tested, only 1,5-diaminobiuret inactivated the transforming principle.

Department of Dentistry

Research in the Department of Dentistry of The Francis Delafield Hospital has been concerned primarily with the clinical and laboratory evaluation of various types of drug therapy.

The Dental Department collaborated with the Department of Bacteriology in a laboratory and clinical investigation of patients treated with Carbomycin. Drs. A. N. Kutscher, J. Piro, E. Zegarelli, S. Lane, and L. Seguin studied Carbomycin blood and urine levels, the effect of Carbomycin on bacteremia, the clinical effectiveness of Carbomycin therapy, and blood chemistry and hematologic findings in patients before and after Carbomycin therapy. Despite clinical effectiveness, blood and urine levels of Carbomycin were found to be extremely low. No side reactions of consequence or evidences of untoward systemic reactions were demonstrated by pre and post therapy blood chemistry and hematology.

In an effort to corroborate the findings of Gladstone with regard to the effectiveness of the Gelfoam biopsy technique in the diagnosis of carcinoma, Drs. Piro, Kutscher, Sproul, and Zegarelli used various methods of collection of Gelfoam sponge biopsy specimens to study the accuracy of the Gelfoam biopsy technique as compared with routine surgical biopsy technique in eleven cases of proven oral and/or head and neck carcinoma. Results demonstrated the lack of justification for using the Gelfoam sponge biopsy technique owing to the relatively high percentage of instances (8 of 11) in which the report of surgical biopsy was definitively positive while the pathological report on Gelfoam sponge biopsy was equivocal or even more often negative.

Efocaine, procaine base dissolved in propylene-glycol, widely heralded as a prolonged acting local anesthetic analgesic of considerable promise, was studied by Drs. Piro, Sproul, Zegarelli, and Kutscher with regard to its safety and effectiveness by injecting Efocaine into the muco-buccal fold of patients following which, at the time of extraction of teeth, biopsies were taken at intervals varying from 2 to 11 days post injection. Histopathological findings were compared

with those obtained in matched areas following saline control injections. A definite plasma cell inflammatory response (with swelling - clinically) was noted following Efocaine injection which was considered to be readily reversible but which, however, contra-indicated the use of Efocaine in areas of degeneration, infection, or pre-existent inflammation.

In order to study the advisability of the intra-oral injection of procaine penicillin and Carbomycin into the oral mucous membranes, Drs. Piro, Sproul, Zegarelli, and Kutscher injected these drugs individually into various areas of the muco-buccal fold of patients and biopsied the areas subsequently. Histopathological findings were compared with those obtained in matched areas following saline control injections. A relatively mild inflammatory reaction of a readily reversible character was observed microscopically but not macroscopically. The sub-mucosal route of injection was therefore found not to be contra-indicated, but its use in degenerated, inflamed and infected areas was considered advisable only after due consideration.

The usefulness of phenoxyethyldimethyl dodecyl ammonium bromide, Bradosol, as an antimonilial agent has been studied by Drs. Kutscher, Seguin, Lewis, Piro, and Zegarelli against 18 pathogenic strains of Monilia albicans in collaboration with the bacteriology laboratory. Bradosol has been found uniformly effective in minimum concentrations of 1:48,000 in inhibiting the growth of Monilia albicans organisms in vitro. Since Bradosol (PDDB) is clinically effective in a .01% solution, has minimum toxicity when used topically at this dilution, and is also a potent anti-bacterial agent, further study of its use in fungal infections, especially in Candida infections, seems highly desirable.

On the basis of the controlled, "double blind" administration of a chlorophyll-containing and a placebo solution to the lesions of 25 consecutive patients with advanced or terminal head and neck carcinoma in which odor had become a constant and prominent aesthetic problem, Drs. Kutscher, Piro, Zegarelli, Chilton and Rankow found chlorophyll to be ineffective as a deodorizing agent.

The effect of an iodine containing mouthwash (differing from Lugol's solution in iodine content both as to chemical composition and concentration) is being compared with Lugol's solution in the bacteriological laboratory in terms of critical killing dilutions, time required to kill micro-organisms, and the effect of the solutions on the activity of antibiotics. No superiority of the new iodine solution (Heliogen) over Lugol's solution was found, and clinical studies comparing these two solutions have similarly been completed without evidence of increased beneficial action of Heliogen.

Department of Medicine

The objective of most of the investigations in the Department of Medicine is clarification of some aspect of the multifaceted problem of disseminated neoplastic disease. This broadly defined approach includes a range of experimental programs from a fundamental study of nucleic acid biosynthesis to the completely pragmatic investigation of methodology in cancer chemotherapy screening. In the past year satisfactory progress has been made in many of the chosen areas of research.

Dr. Alfred Gellhorn and his associates, Dr. Erich Hirschberg, Miss Alice Kells, and Mrs. Marie Lajtha, have carried out studies on five general problems: a) the evaluation of screening techniques in experimental cancer chemotherapy; b) comparative studies of anti-tumor agents *in vivo* and *in vitro*; c) definition of nucleic acid levels in various biological systems and of the effect of carcinostatic agents on these biochemical entities; d) experiments concerned with the biochemistry of differentiation in *Dictyostelium discoideum*; and e) experiments on the carcinogenicity or cocarcinogenicity of tobacco tars.

a) Evaluation of cancer chemotherapy screening techniques - During the past several years, increasing emphasis has been placed on chemotherapy among the approaches to the problem of controlling neoplastic growth. Following a survey on research in this field which was sponsored by the American Cancer Society in 1952, it was recommended that the biological systems commonly employed for screening potential anti-cancer agents be evaluated by comparison with each other and with a variety of other biological test systems. The objective was to determine if improvements could be made, in the empiric search for useful drugs, by employing and correlating a number of screening methods.

A group of about twenty biologists, microbiologists, and biochemists in eight institutions have been conducting a collaborative experiment to study the effects of twenty-seven carefully selected agents on nineteen general biological systems. The organization of this project together with the evaluation and cor-

relation of the results which have been collected has been one of the main functions of this laboratory. A volume detailing the individual data and presenting the correlations and conclusions which may be drawn from them is in preparation.

Of the twenty experimental neoplasms included in this collaborative experiment, three have been studied here. The evaluation of the various agents in mammary adenocarcinoma 755, mouse glioma 26, and the Brown-Pearce tumor in the anterior chamber of the rabbit eye has been completed.

b) Comparative studies of carcinostatic agents in vivo and in vitro - The experiments carried on in close cooperation with Dr. Margaret R. Murray and Mrs. Edith R. Peterson have been briefly mentioned in earlier reports. In these comparative studies during the past year, the Brown-Pearce rabbit tumor and mouse glioma 26 have been employed.

In the Brown-Pearce tumor, 8-azaguanine was an effective inhibitor both in vivo and in vitro, and this effectiveness correlated well with the absence of measurable azaguanine deaminase activity. With two other agents, this good agreement between the animal and tissue culture was not observed. Benzimidazole did not inhibit the tumor in vivo but was fairly effective in vitro; 1,4-dimethane-oxy sulfonylbutane (Myleran) was carcinostatic in the rabbit but had no effect in tissue culture.

Mouse glioma 26, a transplantable brain tumor originally induced by the implantation of a pellet of methylcholanthrene, was exposed to three agents in comparative experiments in vitro and in vivo. 8-Azaguanine was essentially ineffective in both situations; Myleran was inhibitory both in the animal and in tissue culture; 6-mercaptopurine was decidedly carcinostatic in vivo but without significant effect in vitro. The reasons for the different response of tumors to some agents in the body and in tissue culture (benzimidazole and Myleran in the Brown-Pearce tumor; 6-mercaptopurine in glioma 26) are under investigation.

c) The effect of physical and chemical factors on the cellular concentration of nucleic acid - Reference has been made in previous reports of two studies on the effect of anti-cancer agents on regenerating rat liver and particularly on the changes in nucleic acid levels which accompany liver restoration. In collaboration with Dr. Gabriel Godman of the Department of Surgery, a new study has been initiated on the nucleic acid changes in regenerating rabbit muscle following ischemic damage. There is a striking increase in the number of nuclei per mg. wet weight and in the nucleic acid content on a cell or wet weight basis following the trauma. It is hoped that this system will lend itself to a further evaluation of agents which interfere with nucleic acid or protein synthesis.

As part of the continuing program of biochemical characterization of tissues on a cell basis, comparative analyses have been carried out on the nucleic acid content of the Gardner (6C3HED) lymphosarcoma in the solid and ascitic forms. Contrary to expectations, there are wide differences between the two forms, and unusually large variations between succeeding transplant generations of the solid form. Confirmatory assays by the microspectrophotometric technique, a method which does not depend on homogenization of tissues as does the one used in this laboratory, are being undertaken by Dr. David Bloch of the Department of Surgery. The possible occurrence of selection of cells with a particular chromosome constitution in passages from solid to ascitic forms and from the latter back to the former is being investigated.

d) Biochemistry of differentiation in Dictyostelium discoideum - The suitability of this slime mold for studies on differentiation, the general biological process which appears to fail or to be inhibited in neoplastic growth, has been briefly mentioned in last year's report. As part of the collaborative screening program, discussed under a), Dictyostelium has been exposed to a series of anti-cancer and related agents at two distinct stages of morphogenesis and differentiation. In view of the unusually pronounced inhibitory effect of two 2,4-diamino-

pyrimidines on differentiation in this organism, an extensive series of congeners with widely varying antibacterial and antimalarial activity is being tested. In general, the representatives of this class of compounds have pronounced inhibitory activity in this biological system.

Preliminary estimations of nucleic acids, of folic acid, and citrovorum factor, and of other biochemical constituents in this slime mold at various stages of the life cycle are underway to supplement and extend the information obtained by the use of antimetabolites and other biologically active agents.

e) Carcinogenicity or cocarcinogenicity of tobacco tars - The cocarcinogenic properties of croton oil following the application of subthreshold doses of a primary carcinogen, e.g. benzpyrene, is well known. In long-range experiments, it is being determined whether the tars collected from smoke coming from cigarette tobacco "smoked" in a smoking machine have carcinogenic or at least cocarcinogenic activity for the skin of mice. At the present time, over six months after the start of the first experiment, the repeated application of tobacco tars in benzene to the shaved backs of mice has caused no papillomas or carcinomas, but tobacco tars, like croton oil, appear to speed the appearance of skin tumors when a few applications of benzpyrene precede the first application of the tars. It is to be emphasized that the results are not complete enough to warrant any conclusions at this time.

Drs. Zacharias and Renate Dische have been studying the mucopolysaccharides of a variety of tissues. It has been found that the polysaccharide of rabbit bone contains methylpentose. This observation is of great interest because the polysaccharides of rabbit serum contain no methylpentose thus permitting the qualitative distinction between the polymers from serum and bone. It has been known that the polysaccharides are associated with all of the protein fractions. During the past year, the Drs. Dische have fractionated human serum protein according to techniques described at the Physical Chemistry Laboratories at Harvard

(the late Dr. Edwin Cohn) and have characterized the polysaccharides of each protein group.

It was found that the α-, γ-, and β-globulins separated by this procedure differ not only by the concentration of the mucopolysaccharide, but also to a very marked degree by the ratio between the methylpentose and hexose, which in the case of γ-globulin proved to be three to four times as high as in the α- and β-globulins; the two latter also differed in this ratio from each other but the difference was not as pronounced as in the case of γ-globulin. It was furthermore shown that the mucopolysacharide of γ-globulin dissolves almost completely in 80 per cent ethanol containing 5 per cent KOH while in the case of α- and β-globulins most of their mucopolysaccharides remain undissolved under these conditions. It was finally shown that the mucopolysaccharides of various fractions also differ in the speed with which methylpentose can be freed from its link to the other sugars by very mild acid hydrolysis, and this speed can be regarded as characteristic for various fractions and indicative of special structural features of the mucopolysaccharides. The rabbit serum was also fractionated according to method 10, but so far the composition of the mucopolysaccharides of this fraction was not yet determined.

Dr. Emily N. Loeb and Dr. Abbie I. Knowlton have been engaged in adapting existing methods of chemical analyses to the study of rat carcass, muscle and other tissues in order to compare the electrolyte concentrations of these tissues in animals made hypertensive by adrenal steroid therapy and by renal damage through operative procedures.

Work on chemical screening methods for the diagnosis of pheochromocytoma has been continued by Dr. Marcel Goldenberg and his associates.

The influence of Dopa-decarboxylation inhibitors was studied in rats, using 2,5-Dopa. Rat adrenals showed a definite lowering of catecholamine content, most likely due to inhibition of nor-adrenaline and adrenaline synthesis. On the other hand, 3,4-Dopa caused an increase of the catecholamine content of the

adrenal medulla. Since both compounds constitute alternate pathways of tyrosine metabolism the problem is to be studied more extensively.

Dr. Jack Davidson continued studies on the influence of a number of chemical compounds upon the rate of incorporation of radioactive inorganic phosphate into the RNA and DNA of tumor 755 in C57 Bl/6 mice. Standardized studies on 27 compounds have been completed as part of the program on cancer chemotherapy screening methodology mentioned before.

In a series of experiments it was found that a group of compounds exists which do not depress P^{32} incorporation into DNA despite their antitumor activity. The compounds of this group were generally the alkylating agent type and included nitrogen mustard (HN2), Myleran, triethylene melamine, triethylene phosphoramide, and triethylene thiophosphoramide.

Dr. Elliott Osserman has initiated a comprehensive study of serum and urine protein abnormalities in neoplastic disease, particularly multiple myeloma.

A modification of the technique of filter paper electrophoresis has been developed whereby the paper is held in free suspension between two methacrylate frames which form a moisture-saturated chamber. This modification has extended the practicality of an already useful technique by virtue of its ability to increase significantly the degree of resolution of the protein components.

Study has been made of several techniques for the quantitation of the electrophoretically separated protein fractions, including dye-elution, photometry, etc. Although several of these methods provide reproducible quantitative data, there are still formidable methodological limitations which preclude their general use. An extensive survey of special staining techniques for the identification of the several conjugated serum proteins (lipo-glyco-, and mucoproteins) is currently in progress. By these procedures, it has been possible to confirm the fact that a large proportion of the abnormally occurring α-globulins in the serum of patients with advanced neoplastic disease are glyco-proteins.

In conjunction with Dr. Ezra Greenspan of the Department of Medicine, Mt. Sinai Hospital, a study has been made of a method for the turbidimetric estimation of a particular serum fraction which comprises the major moiety of the α-2 plus β-globulins. This simple precipitation technique, originally devised by Dr. Greenspan, may provide useful information concerning this relatively obscure area of the globulin spectrum.

In a consecutive series of 30 cases of multiple myeloma, it has been found that the electrophoretic patterns of the serum and/or the urinary proteins were unique and characteristic in all cases, with no false negative or false positive results to date. The marked electrophoretic homogeneity of the abnormal myeloma globulins distinguishes them from all other pathological hyperglobulinemias. The technique has further proved useful for the serial study of serum proteins in individual patients throughout their course as another method for the objective evaluation of drug therapy in neoplastic disease. In one of six cases of multiple myeloma treated with 6-mercaptopurine, general clinical improvement was shown to be associated with a marked decrease in the abnormal serum globulin. None of the other five myeloma cases so treated demonstrated a comparable protein response.

Dr. George Hyman has studied mechanisms of anemia in patients with neoplastic disease. In 26 patients with malignant epithelial tumors, conventional laboratory tests for hemolysis revealed a moderate increase over normal in 4%, slightly increased hemolysis in 54%, and no significant change in the remainder.

The Ashby technique for study of red cell life-span by differential agglutination was employed by transfusing fresh, normal donor cells in eighteen instances to sixteen of these patients. Moderate to marked acceleration of destruction of donor cells occurred in fifteen patients without any relation to the routine laboratory findings. A repeat study in two patients showed a further increase in the rate of erythrocyte destruction as the disease process progressed.

Investigation of the dynamics of erythropoiesis using radioiron indicated that the majority of these patients fell within normal limits as regards immediate

plasma iron turnover. In addition, red cell iron turnover was normal, and the red cell iron renewal rate normal or increased in the majority of these patients. All the data on iron metabolism, and especially the presence of normal or increased red cell iron renewal rates, supports the thesis that red cell production is normal or increased in these patients.

The studies on red cell production and destruction lead to the conclusion that an increased rate of hemolysis is an important factor in the pathogenesis of the anemia associated with cancer.

In a review of the experience of bone marrow aspiration in patients with cancer, Dr. Hyman noted that tumor cells were found in 10% of the patients. Correlation of the bone marrow study with skeletal x-ray series and other clinical examinations indicated that the bone marrow frequently revealed bone metastasis indetectable by other methods. Investigation of its usefulness as a preoperative survey measure is being carried out.

With Dr. Alfred Gellhorn and Dr. James Wolff, Dr. Hyman carried out a clinical evaluation of 6-mercaptopurine. The drug was found to be useful in the therapy of acute leukemia of children, and to a much lesser extent, in adults. In a small number of patients with reticulum cell sarcoma, limited objective improvement was observed so that further study of mercaptopurine in this disease is warranted. The compound was of no significant value in multiple myeloma, Hodgkin's disease, lymphocytic lymphosarcoma or a variety of epithelial tumors studied.

A number of other clinical research projects have been in progress on the medical wards of The Francis Delafield Hospital. Dr. Daniel Choy, Dr. Jan Mijnssen and Dr. Alfred Gellhorn have been evaluating, by "double blind test", the anti-emetic drug, chlorpromazine. The observations are not complete so that even a preliminary impression is not permissible.

Dr. Donald Tschudy, in collaboration with Dr. Samuel Graff, has studied N^{15} labelled aspartic acid incorporation into the serum and leucocyte protein of

normal and leukemic subjects. Urinary metabolite determinations have also been made. Although the evidence is incomplete, there appears to be a significant difference in the metabolism of aspartic acid between normal and diseased individuals.

Dr. James F. Holland, together with Dr. Alexander Sahagian-Edwards and Miss Ethel Danielson, determined the effect of the chelating agent, ethylene diamine tetraacetic acid, in hypercalcemic patients. They found that although this compound could successfully depress the serum calcium concentration on intravenous administration, the effect was difficult to control and of only brief duration.

Drs. Sahagian-Edwards and Holland reviewed the frequency of adrenal metastases from various primary epithelial tumors. Particular attention was drawn to the fact that complete replacement of these glands occurs leading to the classical manifestations of Addison's disease. Bronchogenic cancer heads the list of tumors which metastasize to the adrenals.

Drs. Alfred Gellhorn and James Holland, together with Dr. Julian B. Herrmann and his associates, completed a clinical evaluation of dihydrotestosterone, Stanalone, in advanced breast cancer. No superiority to testosterone propionate could be found.

During the past year Dr. Helen M. Ranney has continued the study of the occurrence of varieties of hemoglobin by filter paper electrophoresis. This method permits identification of three varieties of hemoglobin: normal adult, sickle cell hemoglobin, and hemoglobin C. The presence of fetal hemoglobin may be suggested by the filter paper method, but is confirmed by the use of alkali denaturation. The combination of sickle cell hemoglobin and hemoglobin C has now been encountered in thirteen patients; all of these patients have a clinical syndrome which is somewhat milder than classical sickle cell anemia. In addition, three patients whose hemoglobin is almost entirely of the "C" variety have been detected -- these permit tentative conclusions about the clinical manifestations of a newly recognized intrinsic defect of the erythrocyte, Hemoglobin C disease. This

disease is characterized by a mild hemolytic anemia, splenomegaly and great numbers of target cells on smears. Studies of the occurrence of these hemoglobins in families confirm the hypothesis that the genes for sickle cell and C hemoglobins are inherited as simple Mendelian dominants. Evidence is accumulating that the genes for sickle and C hemoglobins are alleles.

In conjunction with Dr. S. Waelsch of the Department of Obstetrics and Gynecology, a study utilizing filter paper electrophoresis, of hemoglobin of various strains of mice has been undertaken. Thus far, two types of hemoglobin pattern varying with the strain of mice have been identified.

Together with Drs. I. M. London and J. P. White, a study designed to ascertain the origin of Bence-Jones protein in patients with multiple myeloma has been undertaken. Bone marrow from patients with Bence-Jones proteinuria was incubated in vitro with isotopic glycine; subsequently the Bence-Jones protein may be isolated by immunological methods and its radioactivity determined.

Department of Obstetrics and Gynecology

Cancer research in the Department of Obstetrics and Gynecology, under the general direction of Dr. Howard C. Taylor, has continued to be developed in four particular directions. These may be designated as (1) steroid chemistry and physiology, (2) the study of certain biochemical and metabolic properties of gynecologic tumors in relation to degrees of tissue differentiation, (3) pathological studies, and (4) clinical investigations. The program will be considered under these headings.

I. STUDIES ON THE STEROID HORMONES

A. The Development of Methods

In the laboratories under the supervision of Dr. Seymour Lieberman a large part of the effort has been directed toward the perfection of methods of detection and isolation and toward the discovery of procedures for application to continually smaller quantities of material. Among the technical procedures being worked upon may be noted the following:

1. Application of infra-red spectral analysis to problems of steroid biochemistry (with Dr. Ulrich Weiss, Mrs. Mathilda Furman and Mr. Joseph Zamora).

2. Investigations into methods of synthesis and properties of steroid imidazols (with Dr. Ulrich Weiss).

3. Studies of methods for the synthesis and hydrolysis of steroid glucuronosides (with Mr. Paul Levitan).

4. Methods for the detection of urinary corticoids. The methods perfected in the last year are being applied first to the investigation of the corticoids excreted during pregnancy and by patients with Cushing's disease, adrenal virilism and Kimmelstiel-Wilson Syndrome (with Dr. Jean-Pierre Rosselet and Miss Lillian Overland).

B. Application of Methods of Steroid Analysis

The methods of steroid analysis noted are being used for the investigation of some problems in the metabolism of the steroids and to certain clinical problems

in the cancer field. These may be listed as follows:

1. Investigations into the biosynthesis of steroid hormones by means of perfusion of isolated glands, adrenals, ovaries, testes and placenta, with C^{14}-labeled materials (with Dr. Samuel Solomon, Dr. Raymond Vande Wiele and Miss Alice Lenz).

2. Investigations into the nature and amounts of 17-ketosteroids excreted by a) gonadectomized and adrenalectomized patients treated with cortisone or other steroids, b) pregnant women, c) patients with hirsutism and adrenal virilism, d) patients with Kimmelstiel-Wilson Syndrome (with Dr. Ulrich Weiss, Mrs. Phyllis Garland and Messrs. Elvin DeC. Harper and Gerhard Adam).

3. Investigations of the role of the liver and of the kidney in determining the nature of the excretion of steroid metabolites in the urine of cancer patients. This is to be studied by the administration of exogenous C^{14}-labeled steroids (with Dr. Samuel Solomon and Mr. George Broussalian).

4. The effect of radiotherapy on excretion of 17-ketosteroids, perhaps as a measure of adrenal function, is being observed in a number of cases treated at The Delafield Hospital in cooperation with Dr. Morris M. Gelfand.

C. Studies of Adrenal Hyperplasia

Dr. Joseph Jailer, in collaboration with Dr. Jay J. Gold, a Fellow of the American Cancer Society; Dr. Eleanor Wallace, a Damon Runyon Foundation Fellow, and Dr. Raymond Vande Wiele, a Belgian American Foundation Fellow in Obstetrics and Gynecology, has continued his work on the steroidal aspects of congenital adrenal hyperplasia.

The hypothesis developed in this laboratory, of the existence in cases of adrenal hyperplasia of an enzymatic block which prevents the full hydroxylation of hydrocortisone precursors, has been further substantiated and has received confirmation by a report in the literature from another institution.

Work during the past year has turned to the more intensive study of the problem of hirsutism in women, in particular to correlate precise clinical pictures with urinary steroid patterns. It has been found that there is a group of hirsute women

who excrete large amounts of dehydroisoandrosterone. On cortisone therapy dehydroisoandrosterone disappears from the urine. There is another group of hirsute women whose urinary excretion pattern is normal and whose other symptoms, e.g. amenorrhea, etc., are improved by wedge resection of the ovaries. Consequently, it appears that in the first group the origin of the syndrome is within the adrenal, whereas in the second, it is in the ovary.

Techniques are being developed to study the urinary excretion of "electrocortin" and to separate the components of the human urinary gonadotrophin complex as a basis for next year's program.

II. HISTOCHEMICAL AND BIOCHEMICAL STUDIES IN TUMOR DIFFERENTIATION

The cancer of any organ may be worked upon as a single entity arising in a single mutation from normal cells, or it may be worked upon as a quantitatively varying deviation from the normal. Variability in behavior is common to neoplasms of practically all types, but in some, degrees of malignancy are particularly clearly defined, both with regard to the morphological pattern as well as to their clinical behavior. The papillary tumors of the ovary show unusually well this phenomenon of varying degrees of malignancy or differentiation. In the adenocarcinomas of the endometrium these grades are still distinct, although somewhat less so, while in the squamous cancers of the cervix, "grading" by present techniques is rather unsatisfactory.

The present program is an attempt to study malignancy as a quality by examining various measurable constituents or functions of the tissue and correlating these with the tumor histology and natural history. The program involves histochemical, biochemical, metabolic, and cytological studies.

The initial step is the collection of tumor material and its histological grading by means of classical hematoxylin eosin staining techniques. During the last two years 84 tumors have reached the laboratory, including 20 papillary serous and 13 pseudomucinous ovarian tumors, 13 endometrial carcinomas, 21 epidermoid carcinomas of the cervix and 17 miscellaneous gynecological studies.

A. Histochemistry (Dr. Margaret Long)

Particular attention has been given to the study of the nucleic acids by the use of the Methyl Green-Pyronin Y method. With the RNA of the nucleoli demonstrated by this reaction, nucleolar counts were made showing that their average number per nucleus increased in proportion to the grade of malignancy in the papillary serous tumors of the ovary. With the increase in numbers there was also an observable increase in the average size of the nucleolus and an apparent increase in amount and more irregular distribution of chromosomal and cytoplasmic RNA.

Preparations have also been made for the polysaccharide, glycogen, and the glycoprotein, mucin, but these results are not yet analyzed.

B. Biochemistry (Dr. Dudipala V. N. Reddy)

Biochemical studies have been undertaken upon the nucleic acids and certain enzyme systems in the hope of finding variables that can be correlated with the degree of malignancy.

The ratio of RNA to DNA seemed to be higher in less malignant tumors. These ratios were also found to be related to the polyploidy of the tumors. A limited number of ovarian tumors were fractionated into the cell particulates. The mitochondria were conspicuously absent in all malignant tumors of the ovary. Isolation of pure nuclei and examination of their RNA content has not yet revealed any quantitative difference in the graded tumors studied thus far. The study of the composition of DNA from several ovarian and endometrial tumors has not shown any differences.

The enzyme systems, D-amino acid oxidase, cytochrome oxidase, succinoxidase and arginase were studied in several tumors. The results of D-amino acid oxidase suggest that the benign tumors alone have determinable activity. Succinoxidase activity was totally absent in most of the tumors studied. In the tumors analyzed to date, the arginase activity seems to be higher in the lower grade tumors. The co-factor riboflavin has been determined in a few tumors, but the results are inconclusive.

C. Metabolic and Protein Studies (Dr. Helena deRoetth)

The rate of oxygen uptake of tumor slices in air appears to be related to the grade of malignancy. In general, low $Q_{O_2}^{air}$ values were obtained with benign tumors; the Q_{O_2} was found to increase with increasing malignancy. The Q_{O_2} of the thirteen ovarian tumors received seem to fall into distinct categories which can be related to malignancy. Cervical tumors, with some exceptions, also seem to follow the trend set forth by the ovarian tumors.

The lactic acid production (Q_{LA}^{air}) of the tumors also gives some indication of malignancy although the Q_{LA}^{air} results are not as well defined as the $Q_{O_2}^{air}$ determinations. Benign tumors produce only small amounts of lactic acid while as malignancy progresses increasing amounts of lactic acid are produced.

The preliminary results obtained indicate that when tumor slices are fortified with glucose, they utilize the glucose, but without any apparent increase in Q_{O_2}.

The electrophoretic patterns of grade III tumors differ somewhat from those of the benign tumors. Since only a very few determinations have been run it is not possible to elaborate on this observation at this time.

D. Cellular and Submicro Enzyme Studies

Material from most of these tumors is now being sent to the Department of Biology at New York University where a collaborative investigation is being carried on under the direction of Professor Michael J. Kopac. The work here is directed toward the tissue culture and submicro enzymic studies in single cells and parts of cells. Here too, the object is to find properties of cells which can be correlated with the grades of malignancy of the original tissue.

III. PATHOLOGICAL STUDIES

A number of studies have been completed or are in progress using pathological techniques to study clinical problems.

A. Studies on Radiosensitivity in Squamous Cancer of the Cervix (Dr. Saul B. Gusberg and Dr. Margaret E. Long)

In an effort to find a practical means for making the important distinction between tumors that will respond well and those that will respond badly to radiation, serial biopsies are being taken from the cervix during the course of radiation therapy. These sections as well as tissue smears are being reacted for ribonucleic and desoxyribonucleic acid changes to give a measure of radiosensitivity. Papanicolaou cytological examinations are being made at the same time. Forty-five patients have been studied to date in the analysis of this procedure.

B. Precancerous Lesions

Work has continued, largely under the direction of Dr. Gusberg, on the clinical and pathological aspects of intra-epithelial carcinoma and on adenomatous hyperplasia of the endometrium. In some of this work he has had the assistance of Dr. David B. Moore and of Dr. Harold M. M. Tovell. Dr. Harold Speert has published a paper on the type of endometrium found in cases of cancer of the cervix.

C. Morphology of the Ovary in Gynecological Tumors (Mr. George Rudensky and Mrs. Dorothy Shook)

Evidence from the field of experimental animal tumorigenesis has indicated that the estrogens probably play a significant role in their production. Some clinical reports have also suggested various abnormalities as associated with reproductive tract tumors. With this background, it has seemed reasonable to examine more systematically the ovaries of women with cervical cancer and with other tumors. A method, based on the measurement of theca, granulosa and follicular space in serial sections of the entire ovary has made it possible to calculate the total functioning tissue of ovaries. The technique, which provides a means of comparing the functional capacity of different ovaries, is being used to study especially the ovaries of women with cervical cancer.

IV. CLINICAL STUDIES

Clinical studies include the development of certain improved techniques, the analysis of some series of cases and a carefully organized therapeutic program designed to give answers to certain questions of therapy.

A. Blood Volume Studies in Irradiated Patients

Using a modification of the dye-dilution method for serial estimations of plasma volume, originally introduced by Dr. Albert A. Plentl and himself, Dr. Morrie M. Gelfand has begun the study of the effect on blood volume of massive x-ray therapy directed to the pelvis.

B. Pelvic Venography

Dr. Salim Dalali, working with Dr. Plentl, has made an extensive study of the venous system in the pelvis by means of injected x-ray contrast media. With this study there has been developed a routine method for pelvic venography which has found practical application on a number of occasions.

C. Analysis of Therapeutic Results

Dr. James A. Corscaden and Dr. Tovell have made a report on the clinical management and pathological findings of 247 cases of endometrial cancer treated at The Presbyterian Hospital from 1937 to 1938. Doctors Gusberg, Corscaden and Walter P. Kosar have completed a report on the use of interstitial radium in the treatment of moderately advanced cancer of the cervix.

D. The Therapeutic Program

In the Fall of 1951 a series of meetings was held by a committee of eight, representing the Radiological and Gynecological Services of The Presbyterian and The Francis Delafield Hospitals. The purpose was to draw up a general plan of therapy which would lend consistency to the techniques of therapy being used in the two institutions. The intent of the plan was to insure the patient the best available therapy so far as it was known, and when it was not known, to provide

alternate plans of therapy which might yield eventual conclusions as to the respective functions of surgery and radiation in the field. The need for determining the relative advantage of the different types of high voltage x-ray and of cobalt therapy was also recognized.

The plan, with few modifications, has now been in effect for two and one-half years. It has provided a basic plan for therapy, which has given an orderly approach to the problem, that promises to lead to logically derived at conclusions about indications in therapy.

Department of Pathology

The Pathology Service at The Francis Delafield Hospital carries on active investigative programs with every service of the Hospital correlating the natural history of neoplastic disease with its histopathological characteristics. Dr. Arthur Purdy Stout, Chief of the Pathology Service, has continued his indefatigable studies of all types of tumors. His investigations have contributed invaluable information to the diagnosis and understanding of many types of cancers. During the past year publications summarizing studies of the metastatic spread of breast cancer to the internal mammary lymph nodes, of the natural history of bladder cancer, of juvenile fibromatosis and of cystosarcoma phyllodes have appeared.

The study on breast cancer metastases which was conducted with Drs. J. J. McDonald and C. D. Haagensen is of particular importance because of the frequency of the tumor and of surgical therapy for it. The investigators found that in 66 patients with primary tumors located in the center and inner half of the breast, 41% had internal mammary node metastases; in 38 patients with tumors of the outer half of the breast, only 8% had demonstrable tumor cells in the internal mammary nodes. In the publication listed in the bibliography, the authors discuss the indications for internal mammary and supraclavicular node biopsy, and also critically discuss the indications and limitations of radical chest wall surgery in this disease. In addition, Dr. Stout has edited two fascicles of the Atlas of Tumor Pathology; one on tumors of the soft tissues, the other on tumors of the stomach.

The study of the carcinogenicity of various plastic films in mice and rats undertaken by Drs. B. S. and E. T. Oppenheimer in which Drs. Stout and I. Danishefsky are participating is still continuing. New plastics are being added to the number already investigated and two reports of progress have been published. The search for the carcinogenic agent which may or may not be common to all of these substances is being actively pursued but so far without success. The

Another aspect of the investigation by Dr. Sproul, Dr. Fiala, and Mr. Blutinger has been concerned with the effect of repeated intravenous injections of malonic acid, an inhibitor of succinic dehydrogenase, on the structure and behavior of intact cells and their cytoplasmic components. The mice so treated have shown marked hyperplasia of lymphoid tissue throughout the body and enlarged liver and spleen. Those sacrificed so far present several abnormalities in liver and spleen by ordinary microscopy which are under investigation now. The tissues of those surviving will be subjected to differential centrifugation and electron microscopy studies.

Dr. Fiala is investigating further the working hypothesis that the scarcity of mitochondria in tumor tissue may be of great importance in determining the behavior of the malignant cell. Since the mitochondrial pellet has been shown to be a heterogenous mixture, more exact definition of the deficient particles must be carried out by use of the ultracentrifuge. He is also developing an electrophoretic method in an attempt to characterize the various mitochondrial and other subcellular fractions from the physico-chemical point of view.

Dr. Sproul has collaborated with Dr. Arnold Bachman on a report of the comparison between histologic and x-ray findings in the vertebrae of autopsied patients with malignant disease. The great discrepancy between the amount of tumor in the bone and the ability to demonstrate it radiologically even by x-rays of the excised spine has been the noteworthy finding. This can be explained by the characteristics of the tumor infiltration.

Dr. Sproul has continued a study of the effects of obstruction of the pulmonary lymphatics by metastatic disease on the parenchyma of the lung.

Dr. Sproul collaborated with the Dental Department in studying the tissues of the gum from patients receiving various test substances. These included efocaine, procaine, penicillin, and carbomycin. The efficacy of gelfoam sponge biopsy technique as applied to the oral cavity was also studied and reported.

Mrs. Lucille Seguin has joined the Dental Department in a series of investigations including the effect of carbomycin on bacteremia; the usefulness of bradosol as an antimonilial agent; a comparison by disc sensitivity test of the relative sensitivity of various organisms to carbomycin and erythromycin; and a comparison of heliogen and Lugol's solution as a bacteriocidal mouth wash.

In closing the report of investigative activities of the Pathology Service at The Delafield Hospital, attention should be called to the fact that Dr. Arthur Purdy Stout has reached Columbia University's statutory retirement age and has been awarded the title of Professor Emeritus. Happily for the Institute of Cancer Research, Dr. Stout has agreed to continue his work in the Institute including The Francis Delafield Hospital with the title of Consultant. Inasmuch as Dr. Stout combines the energy of a young man with the wise understanding of neoplastic disease gained by years of broad experience and concentrated study, his associates and the Institution look forward with assurance to many years of invaluable association.

Drs. T. F. Zucker and L. M. Zucker have investigated the factors which modify the action of 2-acetylaminofluorene as a carcinogen.

Instances are known of a modifying action of nutritional factors on chemical carcinogenesis. To study such situations it is desirable and finally necessary to work with so-called purified diets. These are diets in which all the nutritional essentials are given in known amounts as pure substances and the rest of the diet is made up of relatively pure substances with the avoidance of natural foodstuffs which are not pure. On such diets 2-acetylaminofluorene displays a toxic effect which inhibits growth and shortens the life of the animals so that there is frequently not enough time for good development of tumors.

Significant progress has been made in the work of concentrating the factor in natural foodstuffs which has this counter-toxic effect. On a laboratory scale we have succeeded in preparing small amounts of concentrates 50 times as potent as the original material. For further progress larger quantities of material re-

presenting the first concentration steps are needed. We have made arrangements to have such concentrates prepared for us by a drug manufacturer. With regard to the properties of the factor, we have continued to find that it abolishes the toxic early growth inhibition in animals on acetylaminofluorene containing purified diets and that it lessens the liver hyperplasia of the precarcinogenic stage of acetylaminofluorene action.

Department of Pediatrics

During the year, clinical evaluation of chemotherapeutic agents for acute leukemia has continued under the supervision of Dr. James Wolff. Emphasis has been placed upon a study of the effectiveness of Purinethol (6-mercaptopurine) in the treatment of acute childhood leukemia.

Drs. James A. Wolff, Audrey Brown, Frederick Von Hofe and Annie V. Scott, working in the Pediatric Hematology Clinic and on the wards of Babies Hospital, have cooperated in a study of Purinethol with Drs. George Hyman and Alfred Gellhorn, at The Francis Delafield Hospital. Twenty-one children with acute leukemia have been treated with this drug. The majority had had no prior therapy; a few had previously received anti-folic compounds or cortisone. Approximately one-third of the patients have had complete remissions, one-third incomplete remissions, and the remaining third no response to treatment.

Department of Radiology

Dr. Morton Kligerman, together with Miss Elaine G. Rosen and Dr. Edith Quimby, has developed a rotation unit for use with 250-kv. equipment which permits the delivery of cancerocidal doses of x-radiation to volumes of tissue within the body without undue damage to the skin. In studies with Miss Jeanne D. Richmond, Dr. Kligerman has constructed isodose curves in multiple planes about a standard radium application used in the treatment of cancer of the uterine cervix. By applying these curves on the anteroposterior roentgenogram of the patient with the needles in place, the approximate dose at any point in the pelvis can be readily estimated.

Research in radiotherapy, under the direction of Drs. Maurice Lenz and Ruth Guttman, at The Francis Delafield Hospital has been directed toward the clinical evaluation and standardization of cancer treatment by two million volt x-ray and cobalt teletherapy. The increased penetration of this supervoltage radiation as compared to that produced by 200 - 250 K.V. has made it practical to administer larger tumor doses to deep-seated cancers, while reducing the radiation damage to the irradiated skin and intervening tissues. There is little "radiation sickness" with supervoltage and most patients tolerate the treatment better than with the 200 - 250 K.V. type of therapy. The reduced skin reaction and lesser radiation mucositis has facilitated more efficient irradiation of cancers of the head and neck, chest and abdomen. For example, better primary results have been obtained in cancers of the base of the tongue and pharynx than had been previously noted. In cancer of the lung, we have been able to increase the average tumor dose from 4000r in four weeks to 6000r in six weeks, in areas of 10 x 12 cm. or smaller. This has been done with less difficulty and has given better palliative results than when using lower voltages. The consequent radiation pneumonitis has been less severe than that produced by similar dosage when using 200 - 250 K.V. Shrinkage of metastases and lack of serious damage of the normal organ in metastatic cancer of the liver and growth restraint of various malignant tumors

of the upper abdomen by two million volt x-ray therapy has been noted. In cancer of the cervix, stage III and IV, we have seen better palliative results from the increased dosage we have been able to administer by the two million volt and cobalt rotational unit. The chief advantage of rotational cobalt therapy has been noted in fields 8 cm. or less in diameter. Local extensiveness of the cancer and the presence of metastases in many of our patients has, of course, interfered with the efficacy of our purely local method of supervoltage therapy. Improvement from this therapy, therefore, is not reflected as much in an increase of survival figures, as in the better growth restraint and palliation of the cancer patients.

The staff of the Physics Department at The Francis Delafield Hospital, directed by Drs. Karl Braestrup and Alan Lough, has been working on the standardization of isodose curves of the cobalt teletherapy unit. A new light localizing unit has been installed for more accurate localization of the radiation beam.

Dr. Anna Goldfeder is continuing her experiments toward elucidations of the relationship between the rate of growth, metabolic activity and radiosensitivity of malignant tumors, as well as on the significance of the time factor in radiotherapy.

The Diagnostic Radiology Service at The Francis Delafield Hospital, under the direction of Dr. Arnold Bachman, has productively investigated a number of diagnostic problems in clinical neoplastic disease. The radiographic appearance of carcinoma of the colon which extends across the gastro-colic ligament to involve the stomach has been described. The importance of recognizing the correct primary tumor site as being in the colon rather than in the stomach rests on the fact that these colon cancers have a good prognosis following surgery.

With Dr. Dalali, Dr. Bachman has studied the pelvic vein anatomy in living patients by venography. The demonstration of extensive connections between the pelvic veins and the vertebral venous plexuses explains the frequency of pelvic cancer metastases to the vertebral column.

Results on 60 consecutive cases of careful post-mortem x-ray examinations and autopsy studies of the spine have been correlated. The x-ray studies included tomographic analysis. It was shown that x-ray examinations fail to visualize metastatic deposits in slightly more than 50% of the cases. The failures were most frequent where the metastases were intertrabecular and osteoblastic in nature. Tomograms were not more successful than routine radiography in the demonstration of these metastases.

In a study of abnormalities in the lateral roentgenogram of the neck, the appearance of dissociation of the ventricular shadow in hemilaryngeal paralysis was demonstrated and described. By use of tomography a study of the differentiation between functional disturbance of the vocal cords from neoplasms has been conducted. Although the radiographic appearance of the two conditions may simulate each other to a disconcerting degree, the differential diagnosis can be made.

Radiological Research Laboratory

Radiological Physics - Work on the dosimetry of neutrons (both "fast" and "slow") has continued and considerable progress has been made. More reliable tissue equivalent ionization chambers have been constructed. The appraisal of the dose component attributable to gamma rays can now be made better by the use of Teflon ionization chambers in conjunction with the tissue equivalent chambers. Dr. Harald Rossi has devised a new proportional counter method for the same purpose, which may provide a very good solution of the problem in more general terms. However, much work remains to be done before the instrument is fully developed. Mr. Walter Rosenzweig is spending most of his time on this problem.

In recent years there has been considerable uncertainty as to whether the stopping power of water and water vapor for alpha particles was the same (on a mass basis). Mr. Hobart Ellis has carried out a difficult experiment on this subject and finds that there is no difference within the limits of experimental error. This is of great theoretical as well as practical interest.

Mr. William Gross has continued work on the measurement of radioisotopes in absolute terms, using a new method developed in this laboratory. In the case of P^{32} comparisons with measurements made at the Bureau of Standards show agreement within 1%. In the case of S^{35} no intercomparisons have been made because other methods do not yield accurate results. He has also carried out experiments on the energy lost by beta rays in producing ions in different gases. Considerable time has been spent, also, on the calibration of strontium-90 eye applicators and on the measurement of depth doses produced by them.

Mr. Steward Krakauer and Mr. Hugh Byfield have prepared beta ray standards for the calibration of survey instruments by a new method previously developed in this laboratory. A large number of instruments will be calibrated for the AEC with these standards in the near future.

Work on the measurement of the radiation dose in a single cell, produced by radioactive material within it, has been continued by Miss Patricia McClement. This is a difficult problem and many complications have had to be overcome.

Radiobiology - Work under the direction of Dr. Roberts Rugh comprises studies in protection, tissue hydration and fertility changes following whole body x-irradiation of animals. Work in progress may be classified under the headings of protection (adult and fetus) against acute and chronic exposures to x-rays; fetal sensitivity (eyes and gonads), attempts to reduce tissue intolerance of mice to human tumors by prior x-irradiation, and the relation of each of the three phases of estrus to radiosensitivity.

Dr. Ludwig von Sallmann and his group have continued their studies on radiation induced cataracts in rabbits. Some of the experiments were carried out in the Radiation Laboratory of the University of California, this being the only laboratory in which powerful beams of deuterons and alpha particles are available. Drs. John Lawrence and Cornelius Tobias kindly offered their facilities for these experiments and cooperated in the work. The results show definitely that these ionizing radiations are much more effective than x-rays in producing cataracts.

Mr. Bruce Allen and Miss Gwendolyn Wardwell have been studying the anemia produced in rabbits by irradiation of the exteriorized spleen only. This effect was first demonstrated in mice by Mr. Allen last year. The present work is directed towards the elucidation of the mechanism.

Miss Ruth Hill has completed a study on the photoreactivation of T_1 bacteriophage under different conditions and together with Dr. Rossi has proposed a theory to account for the results.

Radioisotope Laboratory - The radioisotope laboratory, under the supervision of Dr. Edith H. Quimby, operates as a receiving, standardizing, and dispensing center for all isotopes used clinically in The Presbyterian Hospital. In addition, the laboratory personnel assist in setting up various isotope research programs throughout the institution.

Therapy with radioactive isotopes - Radioactive iodine continues to be used in treatment of some thyroid cancers as described in last year's report. There are no new developments to report in this program under the leadership of Dr. Virginia Frantz, nor in the use of radioactive phosphorus in leukemia and polycythemia, under Dr. Harold Jacox and Dr. M. Kligerman. The use of radioactive colloidal gold in treatment of pleural effusion or ascites due to carcinomatosis, with Drs. Kligerman and David Habif, has afforded some remarkable instances of palliation. The series of treated patients is continually increasing. No new therapeutic procedures have been developed during the year.

Diagnostic and tracer studies with radioactive isotopes - The routine or service part of the isotope laboratory continues to function as in previous years. Radioactive iodine, both as iodide and labelled human serum albumin, radioactive phosphorus, sodium, and potassium are received, standardized and dispensed to various staff members engaged in research projects.

Radioautographic studies are regularly made on all suspected thyroid cancers at the time of biopsy or surgery. A survey for potential metastases is made at request of the thyroid cancer group; this involves use of a relatively large test dose and point-to-point checking by means of a scintillation counter.

A research problem applicable to cancer which is being carried out in this laboratory is the attempt to identify liver metastases by external counting. This is being done by Dr. Samuel Madell, under the supervision of Drs. Quimby and Kligerman and with the cooperation of the staff of the isotope laboratory. The patient receives 300 μc of iodine-labelled human serum albumin. Twenty-four hours later, counts are made over 42 points marked on the skin through a grid, having the xyphoid process as the point of reference. The mean of the 42 counts is determined, and all counts expressed as a percentage of that mean. These means, for a series of individuals without malignant disease, are used as points of reference. For patients being tested, any value differing by more than two standard deviations

from the corresponding control value is considered abnormal. Data are being accumulated rather slowly but the method shows promise.

Radium and x-ray physics - A grant to Dr. Edith Quimby from the American Cancer Society has supported the work of 2 research assistants on problems connected with radiation dosimetry, Mrs. Victoria de Castro and Miss Beverly Singer.

Work on radiation dosimetry commenced last year on dosage calculation for horizontal rotation therapy has been completed.

A simpler and more direct method for dosage calculation for vertical rotation therapy has been developed and is ready for publication.

The Mayneord contour projector, developed in England some years ago, has been widely employed for study of three-dimensional dose distributions with x-ray beams when circular fields are used. By a simple modification it has been possible to extend use of this instrument to rectangular fields, and to several other special problems.

Department of Surgery

Radical cancer surgery today is far more extensive in its scope than ever before. This is made possible by significant advances in technical procedures, by the contributions of anesthesiology, by the ready availability of potent antibiotics, and by the application of physiological principles to pre and post operative care.

The surgeon is less restricted by the tolerance of his patient for the surgical procedure than by a lack of precise information on the extent of the dissection necessary for cure. Knowledge on this very important issue can best be gained by more facts on the pathways of tumor metastasis. During the past year research on this problem has added valuable insight to help clarify the problem.

In the Laboratory of Surgical Pathology, Dr. Carl Feind, under the supervision of Dr. Raffaele Lattes, has continued a systematic study of the lymphatic spread of malignant tumors of the head and neck. This study utilizes the method of clearing of specimens from radical neck dissections. So far, approximately 100 cases have been studied. The careful charting of the involved and uninvolved lymph nodes on appropriate topographic drawings has increased the knowledge of the normal and alternate pathways of lymph node spread from cancer of the head and neck. The average number of lymph nodes found in each case was 63. The incidence of metastases in so-called prophylactic or elective neck dissections has been 46 per cent. This fact alone will certainly influence future surgical handling of these cases.

The study of axillary and internal mammary lymph node metastases from carcinoma of the breast, also using the clearing method, has continued. This was initiated by Dr. John Pickren, who is at present in the United States Navy. More than 200 cases of carcinoma of the breast have been studied; the average number of lymph nodes found in the axillary fat was 35.4. The incidence of metastases was 56 per cent. This study has helped in establishing more accurate prognosis following radical mastectomy for carcinoma of the breast.

The lymph nodes from 29 axillary dissections, which had originally been reported as negative for cancer on routine histologic study, were studied in serial sections by Dr. Pickren. With this method, microscopic metastatic foci were discovered in four (14%) of these cases which had been reported originally as negative.

Dr. Robert Grinnell's studies on the distribution of metastases from carcinoma of the colon and rectum have contributed greatly to rational intestinal cancer surgery and are presented in some detail.

(1.) During the past year a clinical study of all cases of squamous cell carcinoma of the anus admitted to the hospital since 1916 has been carried out. Forty-nine cases were found, which represented 1.8 per cent of all anal and large bowel carcinomas admitted during this period. Especial study was given to the routes of spread of these tumors and the results of treatment. Local spread to nearby organs, such as the prostate, vagina and bladder, was infrequent and occurred only late in the disease. Spread by the blood stream was rare and occurred in only two patients, both of whom had liver metastases. Spread by the lymphatics to the regional nodes was frequent. Upward spread to the nodes along the inferior mesenteric artery was found in seven of 25 resections, an incidence of 28 per cent. Metastasis to the inguinal nodes was infrequent, occurring in only four patients and only late in the disease. Upward spread to the perirectal and mesocolic nodes and lateral spread to the pelvic nodes appeared to be more important.

Before 1943, patients treated for cure were treated by both surgery and radiation. Since 1943, radiation has been used only as a palliative measure. In the 38 patients available for 5 year follow-up analysis, the absolute 5 year survival and cure rates were 36.8 and 31.6 per cent respectively. Twenty-nine of these patients were treated with hope of possible cure, 21 by surgery and 8 by radiation. Two of those treated by radiation were alive after 5 years. The relative 5 year survival and cure rates in the surgically treated patients was

52.4 and 47.6 per cent. Both the absolute and relative survival and cure rates compare favorably with those previously reported from this hospital for carcinoma of the rectum.

Since it is questionable whether radiation can destroy cancer in the nodes, such as the perirectal and mesocolic, it is believed that radical surgery by abdominoperineal resection offers the best chance of cure. As local excision does not remove the regional lymph nodes, it is not recommended even in small tumors which often have lymph node metastases. Radical surgery can be expected to show still better results in the future.

(2.) In the past several years a study has also been carried out on the distal intramural spread of carcinomas of the rectum and rectosigmoid. This is of clinical importance in the controversy as to whether anterior resection or abdominoperineal resection should be the operation of choice in carcinomas of the recto-sigmoid, and if anterior resection is carried out, what should be considered to be an adequate margin of bowel distal to the tumor. A total of 126 specimens of carcinoma of the lower colon and rectum were studied, especial attention being given to 93 which lay from 5-18 cm. above the dentate line, which is the area of bowel most concerned in this controversy. Blocks of tissue, 1 cm. in width, were cut below the tumor for a distance of at least 3 cm. Four sections were taken from the upper surface of each at an interval of 250 microns. If more sections were desired the whole block was sectioned and further blocks taken if needed.

Retrograde intramural spread from 1-7 cm. in extent was found in 18 out of 93 tumors lying from 5-18 cm. above the dentate line. It occurred in 9, or over one-half of the 17 tumors removed as palliative resections, and in 9, or 12 per cent of 76 tumors removed as curative resections. The maximum distal spread in a curative resection in this group was 4 cm., 2 cm. in 5 tumors lying less than 5 cm. from the anus, and 1.5 cm. in 28 tumors above the 18 cm. level.

Retrograde mural spread was chiefly by way of the lymphatics of the subserosa and appeared to be caused by the blockage of upward lymph flow by node metastases with subsequent lymphatic permeation of tumor cells downward. Occasionally it occurred as the result of permeation of a vein and rarely from direct extension of tumor cells in the muscularis. Distal spread was most common in advanced tumors of high grade malignancy, with many node metastases, frequent vein invasion, and annular involvement of the bowels. It was most frequent in palliative resections.

In curative resections distal spread did not appear to have great clinical significance. A margin of 5 cm. of uninvolved bowel would have been adequate in every case although more is preferable.

Dr. Virginia Kneeland Frantz and her associates have continued work in thyroid cancer including detailed study of specimens from radical dissections and serial sections to establish possible histological variations in primary tumors and their metastases. In collaboration with Drs. Morton Kligerman and Edith Quimby, Dr. Frantz has embarked on a long-term experimental investigation of thyroid carcinogenesis by external irradiation and internal radiation from radioiodine.

Research

The newly organized Histochemistry Laboratory under the direction of Dr. Gabriel Godman has had an active and productive year in collaborative studies with other departments and also in experiments initiated in the Laboratory.

Dr. D. Bloch investigated the cytophotometry of Feulgen and methyl-green preparations. Employing the visible range of the spectrum he found a nearly perfect correspondence of class distributions indicating that the sugar and phosphate moieties of DNA are formed at about the same time. 2,6-Diaminopurine, thiouracil, 5-nitrouracil, 8-azaguanine, azadenine, benzimidazole, 5-aminouracil, and myleran, did not appreciably influence the formation of DNA in surviving cells. The effect of colchicine, however, on the distribution of DNA "classes" was marked; there was a progressive increase of polyploid interphase nuclei (to 16 C) with time of exposure up to 26 hours, owing to the reversion of mitotically arrested cells to

interphase. DNA synthesis per se proceeded at nearly the same rate as normally, in spite of mitotic arrest.

As a prerequisite to relative quantitation of basic nuclear protein of the histone type, Drs. Bloch and Godman investigated and validated for microspectrophotometric use a new dye-binding method with fast green. A technique was elaborated permitting Feulgen staining for DNA determination to be followed by fast green staining for histone determination in the same cell. Direct cytophotometric comparisons of DNA with histone in identical nuclei of growing tissues gave a very similar distribution of "classes" for both. The data indicate that the syntheses of DNA and histone proceed approximately simultaneously and relatedly, although it remains to be seen whether they are interdependent.

During observations on the growth of sarcoblasts from striated muscle in tissue culture, it was found that colchicine in concentrations, even below those causing mitotic arrest,caused disruption, expansion, decreased viscosity, and loss of birefringence of sarcoblasts, which, however, remained viable. This was related to the known effects of colchicine on the mitotic spindle and the cytoplasm, and is thought to represent in each case an interference by colchicine with oriented chains of contractile protein micelles: systems important in many cell functions.

Dr. Godman found cytochemical differences in lipid, carbonyl group and phosphatase content between the free histiocytic cells and the muscle fibers from which they take origin both in vivo and in tissue culture.

Dr. C. Bassett studied the morphological characteristics of modulating osteoblasts in vitro; at some stage in their modulation in vitro such cells exhibit marked cytoplasmic alkaline phosphatase activity which might be correlated with their osteogenic potency.

In addition to the projects described above, Drs. Godman and Bloch are active in the following collaborative studies:

1) Microspectrophotometric measurement of DNA and nuclear protein synthesis in tissue cultured cells, with reference to the mutual relations of these substances in the course of the mitotic cycle, and to the effects of certain mitotic poisons and anti-metabolites on their syntheses. In this work, DNA and protein synthesis in various nonmitotic forms of cell division (meiosis; amitosis) in tumors, and the effects of certain drugs, remain to be investigated. An effort is being made to determine whether the processes concerned with histone synthesis can be dissociated from those connected with DNA.

2) The histochemistry of regeneration and differentiation of striated muscle, *in vivo* and *in vitro*. A correlation of the presence, relative amounts and localization of nucleic acids, myohemoglobin, lipids, glycogen and certain enzymes with the successive morphological stages of sarcoblast differentiation is being made. Dr. E. Hirschberg of the Department of Biochemistry is collaborating in the study of the nucleic acids.

3) Nucleic acid and protein synthesis in regenerating spinal ganglion neurones in tissue culture. This project is being carried out jointly with Dr. M. R. Murray, Mrs. E. Peterson, and Mrs. A. Deitch of the Laboratory for Cell Physiology.

4) Chemistry of the production of mucopolysaccharides by various mesenchymal cells, in mass tissue culture. This project is being carried out with the Rheumatic Diseases Study Group of the Department of Medicine (Drs. C. Ragan, K. Meyer, H. Grossfeld). The types and quantities of mucopolysaccharide produced *in vitro*, the substrates, enzyme systems involved, and the effects of hormones and other agents on this system are under investigation.

5) The growth of osteoblasts in mass tissue cultures is being evaluated jointly with Dr. Bassett of the Department of Orthopaedic Surgery, as a possible means of bone graft procurement. The morphological growth characteristics of bone-forming cells and correlated cytochemistry are under investigation.

The Laboratory for Cell Physiology is under the direction of Dr. Margaret R. Murray. The over-all purpose of this laboratory is to investigate a variety of problems at the cellular level, using the methods of tissue culture. The cancer research performed here has followed three main lines of interest: A. Chemotherapy and metabolism of tumors, especially those of human origin, B. The mechanism of mitosis, approached through mitotic poisons and their inhibitors, C. Development of criteria for classification and diagnosis of human tumors.

A. Since metabolic pathways differ, between species as closely related as the rat and the mouse, animal screening programs can offer only preliminary data on carcinolytic agents for human use. Tissue culture, especially when correlated with other analytical methods, affords a valuable means of bridging this gap. For several years, with the collaboration if Drs. J. Lawrence Pool, Alfred Gellhorn and Erich Hirschberg, Research Associate Edith Peterson has been investigating potential chemotherapeutic agents for the human glioblastoma. Of 9 compounds tested, 5 have been found to be inhibitory in some degree to this neoplasm in vitro. These are 8-azaguanine, benzimidazole, 5(or 6-)-chlorbenzimidazole, 6-mercaptopurine and 1,4-dimethanesulfonyloxybutane (Haddow's GT41). A transmissible glioblastoma of mice (methylcholanthrene-induced) is being tested also; and this is found to differ somewhat from the human spontaneous tumors in its susceptibility to these agents. In lesser degree, the human tumors (from 12 different individuals) differ among themselves.

B. Determinations of the mitotic coefficient (ratio of dividing to resting cells) and of phase percentages among the dividing cell population, in a biological system involving the adult rat fibroblast in vitro, have been standardized as a quantitative approach to the mechanism of mitosis. With the collaboration of Dr. Erwin Chargaff, Research Assistant Helena Benitez has shown by a series of rigorously controlled experiments within this biological system that the effects of colchicine, generally regarded as a spindle poison, can be

antagonized significantly (50-75 per cent) by three compounds widely different in chemical structure. These are meso-inositol (one of the B-vitamins), adenosine triphosphate (a link in the actomyosin system), and tropolone (a simple heptane analog of colchicine itself). The efficacy of this variety of inhibitors seems to underline the concept that nuclear division results from a chain of reactions which may be interrupted at manifold points, and to suggest that "unitarian" theories of mitotic mechanics should be re-assessed as to their emphasis.

C. The cultivation of rare and little understood human tumors as they come provides a gradual but significant accretion of morphological and physiological information which may be used for many purposes. With the collaboration of Drs. A. P. Stout, R. Lattes, N. Lane, and M. Melicow, the usually benign ependymoma has been shown to be capable of metastasizing, a neurilemoma has been found in the lung (a hitherto unreported site), and the phaeochromocytoma has been found to produce a cell-type closely resembling that of the sympathicoblastoma.

Dr. Daniel M. Shapiro, together with Dr. L. S. Dietrich of the Department of Biochemistry, and Dr. Maurice Shils of the School of Public Health, have continued studies toward the two principal objectives of their research program: one, to explore the feasibility of utilizing quantitative biochemical differences between normal and neoplastic cells as an approach to cancer chemotherapy; and, two, to study the possibility of eradicating neoplastic diseases by the simultaneous or sequential administration of combinations of chemical compounds selected upon this basis.

Encouraging data in support of the first objective was obtained through study of pyridoxine metabolism in a transplantable mammary adenocarcinoma (755) and the normal tissues of its host, the C57 black mouse. In vitro studies demonstrated that the low pyridoxine content of the 755 tumor renders these malignant cells vulnerable to vitamin B_6 antagonist therapy (desoxypyridoxine), and that testosterone potentiates the carcinostatic activity of desoxypyridoxine by

further lowering the vitamin B_6 content of the tumor. More recent studies now reveal that testosterone, although having no apparent effect upon the activity of vitamin B_6-containing enzyme systems per se, markedly potentiates the sensitivity of glutamic-alanine transaminase and dopa decarboxylase to antagonism by desoxypyridoxine. This phenomenon was observed to occur in all tissues studied (liver, kidney, heart, lung, and tumor).

The results of certain biochemical studies present the possibility that vitamin antagonists may be synthesized to function solely as specific anti-enzymes, and not as antagonists for all the biochemical functions of the vitamin. For example, only two (glutamic-aspartic transaminase and dopa decarboxylase) out of three of these vitamin B_6-containing enzymes were found to be inhibited by desoxypyridoxine therapy. Furthermore, only one of two riboflavin antagonists affected the activity of a flavo-enzyme, xanthine oxidase. These data present the possibility that vitamin antagonists may be useful as highly precise chemotherapeutic agents, particularly in the presence of a normal dietary intake.

Progress in the direction of achieving the second objective of this research program has been documented by the addition of the riboflavin antagonist, 6,7-dimethyl-9-hydroxyethyl isoalloxazine (U 2113), to the triple combination of 8-azaguanine plus desoxypyridoxine plus testosterone. The latter triple combination has been previously shown to exert greater carcinostatic effects against the 755 tumor than any of the possible smaller combinations of the three compounds. The results of experiments in which all four compounds are given daily, reveal that each drug is contributing to the striking carcinostatic results obtained. Moreover, preliminary results with eight compound combination chemotherapy demonstrate that multi-combination chemotherapy can transcend from carcinostatic to carcinocidal activity.

Work is currently in progress to clearly define the points of action of the above-mentioned riboflavin analogue (U-2113). Total riboflavin, FAD, and FMN concentrations are being determined in the 755 tumor and the normal tissues of its host. In addition, the activity of certain flavoenzymes following the in vivo administration of U-2113 is under study.

Additional hormone-enzyme relationships have come under study other than those already indicated. It would appear that mouse liver xanthine oxidase is either directly or indirectly under hormone control, inasmuch as both cortisone and histamine markedly elevate liver xanthine oxidase activity. An indication that this effect may be indirect is that xanthine administration produces a similar response.

The necessity for determining enzymatic activity in tissues of low enzymatic concentration, such as tumor, has led to the development of more specific micromethods. Refined procedures have been developed for measuring dopa decarboxylase and xanthine oxidase activity.

Drs. C. D. Haagensen, Leon Dmochowski, and Daniel Moore have continued research on mouse mammary cancer. The mouse colony which supplies test animals and material for investigations has a current population of 14,000 mice: RIII high- and RIIIb low-cancer, A high- and Ax low-cancer, C57 low and C57+ agent-harboring strains. Separate dairy colonies of these strains are also available, and 500 C57 low-cancer-strain females mated with RIIIb males which serve as a source of (C57xRIIIb) F1 hybrids (test mice). During the past year 7425 litters have been raised, comprising 39,490 mice. The colony for breeding hybrids is being enlarged to obtain an average of 300 hybrid females a week.

Multiple biological tests of material from suitable test mice of a single strain have been carried out in an attempt to determine the best source of the milk agent. The following material from RIII Paris high-breast-cancer-strain mice has been tested: whole milk; milk clarified by centrifugation at 600 times gravity for 10 minutes and freed of the resulting fatty layer and deposit; spon-

taneous breast tumor tissues as a 10% extract in 0.25 M sucrose clarified by centrifugation at 600 times gravity for 20 minutes; lyophilized milk and lyophilized spontaneous tumor tissue. The tested material was used in serial dilutions ranging from 10^{-3} to 10^{-10} which were made in 0.25 M sucrose containing C57 mouse serum in 1:100 proportion to prevent possible loss of activity through adherence to glassware. The tests were carried out in 1750 test mice. Tumors have been obtained in all dilutions of the five tested materials, but it is too early to determine which will prove to be the best source of the milk agent suitable for further study and also how certain treatments influence the tumor-inducing activity.

In an attempt to ascertain whether casein or lipoprotein particles carry the milk agent, RIII high-cancer-strain milk has been fractionated into casein and lipoprotein fractions by differential centrifugation, following preliminary clearing at 600 x g for one hour. Electron microscopy and biological tests of these fractions in serial dilutions ranging from 10^{-2} to 10^{-6} have been carried out. In the electron microscope the casein as well as the lipoprotein fractions show the presence of spherical particles varying in size from 2500 A^{o} to less than 300 A^{o} in diameter and differing little in appearance; the lipoprotein particles perhaps appearing more flat and less dense. So far, 174 test animals have been used. The experiment will be repeated until at least 40 test animals will have been injected with each dilution of the two fractions.

The action of prostate phosphatase and snake venom diesterase on casein particles has been investigated. These two enzymes have been obtained through the generosity of Dr. Gertrude E. Perlmann from whose work (J. Am. Chem. Soc. 74, 3191, 1952) it was already known that casein is dephosphorylated by the action of prostate phosphatase. Electron microscopy of casein particles treated with these two enzymes at suitable pH for periods of 2-24 hours has been carried out. The casein particles do not appear to undergo any appreciable change in numbers or appearance

following this treatment. On the assumption that casein particles carry at least part of the activity, biological tests will be carried out on treated particles to find out if any diminution of tumor-inducing activity takes place following treatment with these enzymes. Similar tests will be carried out on casein particles treated with disodium versanate, as in preliminary experiments it has been observed that suspensions of casein particles become clarified following this treatment and cannot be seen in the electron microscope.

The study of the electrophoretic mobility of extracts of RIII Paris tumors, dried, trypsinized, and differentially centrifuged, has been continued, and a fraction moving ahead of any visible boundary and containing 119 γg of N per ml. has been tested in mice in dilutions varying from 10^{-5} to 10^{-11} as well as non-moving fraction containing 155 γg of N per ml. Additional experiments on these lines will be done as soon as test animals become available. These fractions are known to contain spherical particles of 300 A° average diameter, and it is hoped in this way to ascertain the activity of such particles and their homogeneity.

Another series of experiments has been started in order to establish the size of particles showing the highest-tumor-inducing activity. RIII high-cancer-strain milk in a 10% suspension in nutrient broth following preliminary centrifugation at 600 times gravity for 20 minutes was filtered first through collodion membranes of 7800 A° average pore diameter. Part of the filtrate in dilutions of 10^{-2} and 10^{-4} was tested in mice and the rest filtered through 1600 A° and 1000 A° average pore diameter membranes. Filtrates through these two other membranes have also been tested in similar dilutions in the test animals. Additional experiments through the same as well as other diameter size membranes are now being carried out so as to obtain a comparison of tumor-inducing activity of various size particles from milk as well as from tumor tissue.

Electron microscopic studies have been carried out on thin sections of suitably prepared mammary tumors from agent-containing and agent-free strains, some

of them isogenic. So far, 37 breast tumors have been examined; 18 tumors from high- and 19 tumors from low-cancer-strain mice were examined in 840 sections. Spherical particles with an average diameter varying from 500 to 1500 A° have been observed in the cytoplasm, frequently in the intracellular spaces but not in the nucleus. In some sections the particles showed internal structure composed of a dense center surrounded by a lighter zone which in turn was surrounded by what appears to be a sharply delineated membrane. The particles have been found in seven out of ten RIII Columbia, in all four RIII Leeds strain, and in three out of four C3H high-cancer strain tumors. They have not been observed in six RIIIb agent-free strain tumors, but have been found in two out of five C3Hf (Bethesda) agent-free tumors, supplied by Dr. W. E. Heston. Similar particles have also been found in two out of eight C3H (Roscoe B. Jackson Memorial Laboratory) low-cancer-strain tumors, sent by Dr. C. C. Little. All low-cancer strain tumors have at the same time been tested biologically in susceptible mice. In addition, lactating mammary gland tissue from RIII high-cancer-strain, C57 Black low-cancer-strain, and from Ax agent-free mice have been studied. Particles, presumably casein or lipoprotein, similar in appearance with at least some of the particles seen in the sections of breast tumors, have been found in all the examined sections of these glands. Additional low-cancer-strain tumors as well as tumors induced in low-cancer-strain mice by the agent are being studied. It will be important to correlate these findings with the results of the biological tests of these tumors. Preliminary attempts to differentiate the particles seen in tumor sections from those of casein have been unsuccessful because disodium versanate, which destroyed fresh casein particles, failed to have a sirilar action on casein particles fixed with osmium tetroxide. Other means will have to be resorted to.

Dr. Michael Heidelberger has fractionated milk from RIIIb agent-free strain mice and has used the fractions to absorb the rabbit antisera against the RIII strain milk factor as isolated by Dr. Samuel Graff. These absorbed sera are now being tested against milk factor preparations and against agent-containing and

agent-free milk. The first tests of so absorbed sera showed strong precipitation with RIII strain milk factor preparations, indicating that there is some antigen present in the milk factor preparations which is not present in the factor-free milk of the same strain of mice.

Department of Urology

Dr. Perry B. Hudson and his associates, Drs. A. L. Firkle, J. M. Reiner, M. Lombardo, M. London, T. D. Price, and K. K. Tsuboi, have continued their broad studies on problems of genito-urinary cancers.

During the last year, laboratory studies on the prostatic acid phosphatase enzymes have shown that these substances are specific proteins probably foreign to human serum except in those instances in which carcinoma has metastasized. A tentative correlation between the extreme elevation of serum prostatic acid phosphatase and alteration in hepatic function has been achieved. The efforts directed toward the isolation of the enzyme have recently been successful. The phenomenon of normal, low acid phosphatase values in patients with known metastatic carcinoma has been described. Laboratory studies have demonstrated that the enzyme is denatured mainly by heat, and this principle has been employed clinically for experiments which produce an increase in the circulating enzyme by lowering the body temperature. Observations made in cases of metastatic cancer patients suffering urinary tract infections with fever have confirmed this laboratory hypothesis regarding the behavior of the enzyme in response to heat. The studies in enzyme kinetics have been continued. A neutral phosphatase has been studied, human red cell phosphomonoesterase, and the stabilization and inhibition characteristics have been explored.

Theoretical studies have been made in the laboratory on the mechanism of uremia, and artificially induced uremia has been produced in dogs for this purpose. Techniques for the purification of uricase have been developed, and possible mechanisms for the potential enzymatic control of uric acid metabolism have been postulated. The investigations into methodology and the acquisition of factual and theoretical information precede a clinical study of uremic patients which will shortly be initiated.

The steroid chemistry problems investigated have included those relating to the extraction of steroids from human blood. These experiments have been made

possible by a simplified technique for cannulating the central adrenal vein which was developed during the last two years. The isolation of specific steroid substances has been achieved. The principle of dialysis coupled with continuous extraction has been modified to make this isolation practicable. Recoveries of between 90 and 100 per cent of deoxycorticosterone, cortisone, hydrocortisone, androstenedione and other steroids from blood have been made. The mechanism of urinary steroid excretion following hormonal depletion surgery has been studied in connection with bilateral or total adrenalectomy for advanced mammary cancer. A clinical evaluation of the effective dosage levels of the monoammonium salt of glycyrrhizinic acid has been made as part of an appraisal of the drug in adrenalectomized patients.

In the laboratories devoted to the study of nucleic acid metabolism, vascular perfusion and the use of radioactive tracer compounds have been employed in studies on the biosynthesis of nucleic acid in excised human kidney and testis. A consistent pattern of isotope distribution has been found in the nuclear and cytoplasmic pentosenucleic acid of these organs. The metabolic stability of the nucleic acids continues to be studied. Concurrent with the isotope studies, new methods for evaluating the radiochemical purity of tracer compounds have been developed. Techniques for the visualization and identification of compounds have been so modified that nucleotides, nucleosides and bases from nucleic acid can be identified on paper chromatograms. Improvement in instrumentation have accompanied this work in paper chromatography.

The clinical evaluation of the effectiveness of several drugs has been completed. The use of nisentil hydrochloride as an analgesic-narcotic in the preparation of patients for minor surgical procedures has been iritiated and completed. The use of glycyrrhizin as a maintenance drug in patients following total adrenalectomy has been explored. The oral dosage level of diethylstilbestrol has been studied, and the effectiveness of large oral dosages in advanced prostate carcinoma has been demonstrated.

The evaluation of surgical techniques for the early detection of unsuspected prostatic adenocarcinoma has been continued. After it was demonstrated that transurethral resection biopsy techniques have little value in the detection of unsuspected carcinoma, the technique has been abandoned except for the documentation of known metastatic cancer. Following an appraisal of the effectiveness of the diagnostic adequacy of the needle and open perineal biopsy techniques in 200 consecutive patients, the value of the open perineal biopsy for the detection of unsuspected adenocarcinoma was indicated. The effectiveness of this technique was later demonstrated in a study of 300 consecutive patients. Mortality following radical surgery has been appraised, and a constantly decreasing death rate following such procedures has been noted.

The study of unsuspected adenocarcinoma in the aging male population continues with the cooperation of the Department of Welfare of the City of New York. Approximately 250 men enter The Francis Delafield Hospital each year, and there have been approximately 500 admissions of this sort to date. In approximately 400 instances, complete clinical evaluations of these men have been obtained, and early cancer has been detected in approximately 13% of the cases. In order to obtain closer follow-up of these and other patients, a research service unit has been established and a biostatistician employed as a member of the staff.

In addition to these activities, which have been under the direct supervision of the Department of Urology, the research group headed by Dr. P. B. Hudson has engaged in activities which have been collaborative efforts with other departments in The Francis Delafield Hospital. In association with the Department of Cardiology, the earlier work on the cardiac risk of patients with genito-urinary cancer has been completed. In conjunction with the Department of Neurosurgery, investigations of new surgical techniques designed to alleviate long term pain in cases of metastatic carcinoma have begun. The liaison with the Department of Pathology continues to be a close one, and work on the microscopic differential tissue diagnosis of carcinoma continues.

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George A. Hyman

SCIENTIFIC REPORT OF THE INSTITUTE OF CANCER RESEARCH

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COLLEGE OF PHYSICIANS AND SURGEONS

COLUMBIA UNIVERSITY, NEW YORK

Scientific Report of the Institute of Cancer Research

1954 - 1955

College of Physicians and Surgeons

Columbia University, New York

Presented by the Cancer Research Coordinating Commitee:

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Introduction

This volume is dedicated to the foundations and individuals who have made possible the research of this Institute through financial support. Equally, it is a summarizing report to scientific and clinical colleagues throughout the world who are aleo devoting their interests and efforts to the study of abnormal growth. Each year it is reassuring and encouraging to note that we at Columbia University are a part of a cooperative undertaking with the public and scientific communities to control the multitudinous diseases known as cancer. Each year, it seems to us, the work that has been done by the thousands of investigators cuts this awesome pathological phenomenon down to a size that is more easily comprehended. Thus the recognition of the probable importance of environmental factors in the genesis of lung and cervix cancer provides a tremendous stimulus for productive research which may lead to prevention of a significant proportion of these tumors; the greater understanding of mechanisms of anemia in neoplastic disease removes this characteristic and distressing manifestation from the realm of mystery to the laboratory and clinic for clearly defined study; the discovery of the frequency of internal mammary node metastases in apparently operable breast cancer explains why many patients are not cured even though the local lesion has been completely removed; these and many other examples might be cited to illustrate the point.

Advances in cancer research parallel greater understanding in all areas of general knowledge and more specifically in biological phenomena. It is for this reason that support, encouragement and recognition are given to investigations of biological events which are not immediately directed toward clinical applications. At the Institute of Cancer Research this category is well represented in laboratories of Biochemistry, Cell Physiology, Histochemistry, Physics and Biology. During the past year there have also been a number of studies which have immediate implications for the patient with neoplastic disease. These include:

The paper electrophoretic analysis of serum and urine has been established as a specific diagnostic procedure in multiple myeloma; it has been possible by

the use of supervoltage radiotherapy to eradicate bronchogenic cancer without significant damage to the surrounding normal lung parenchyma; it has been demonstrated that the erythrocytes from patients with cancer have a normal life span when transfused to a normal recipient thereby further indicating that a hemolytic factor is most important in the genesis of the anemia of cancer; observations have been made on the malignant human brain tumor, glioblastoma multiforme, which indicate that a chemotherapeutic attack is well within the realm of possibility.

In the succeeding pages the research alluded to will be briefly summarized. Amplification of the work of the one hundred and fifty-two investigators can be achieved in part by reading the appropriate references among the one hundred and forty-eight publications cited in the bibliography of this year.

Alfred Gellhorn, M.D.

June, 1955

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Biochemistry

As in past years, the members of the Department of Biochemistry have studied many facets of metabolism and of normal and abnormal growth. This report will touch only upon those investigations which have had preponderant support from cancer funds.

Dr. Zacharias Dische has continued his studies of the mucopolysaccharides of serum proteins. The mucoid split off from human serum glycoproteins by mild alkaline hydrolysis was analyzed in detail; at least half of this material consists of chains of at least two alternating oligosaccharides, one of which contains fucose. The aldehydic end group of every oligosaccharide molecule is combined with an amino acid, the nature of which is under investigation.

In conjunction with Dr. Alfred Gollhorn of the Department of Medicine, Dr. Dische has undertaken a search for possible correlations between certain types of disease, particularly malignant tumors, and changes in the neutral mucopolysaccharides of the plasma proteins. Variations of the distribution of these carbohydrates between individual plasma protein fractions as well as in the content of fucose and in certain structural factors are under investigation. Of particular interest is the determination of the relative rates of liberation of fucose and hexose, a relationship which is characteristic for every one of the protein fractions and which is altered significantly in the plasma of cancer patients.

Dr. Stephen Zamenhof has continued, in collaboration with Dr. Hattie E. Alexander of the Department of Pediatrics, his studies on the resistance of the transforming principle (heredity determinants, DNA) to mutagenic, carcinogenic, and carcinostatic agents. It was found that all strong mutagens tested and some of the carcinogenic and carcinostatic agents produce unstabilization of the transforming principle prior to actual inactivation. This phenomenon is believed to be analogous to gene unstabilization prior to mutation. In general, the parallelism between the phenomenon of gene mutation and the inactivation of

heredity determinants (transforming principle) by mutagens has been established, lending support to the hypothesis that the substance undergoing a change in the gene mutation is DNA.

In another project, Dr. Zamenhof has studied the introduction of unnatural (halogenated) pyrimidines into DNA of E. coli. The correlations between the amount of thymine replacement on one side and the size of the halogen substituent or the properties of the bacterial strain on the other side have been studied. The cells with such unnatural DNA were found to have unchanged genotype. It was noted that whereas replacement of more than ten per cent of methyl groups in DNA thymine by bromine or chlorine does not result in either inactivation or demonstrable mutation; removal of less than one-half per cent of total amino groups and/or hypothetical hydrogen bonds to these groups results in total inactivation of DNA.

The Biochemistry Laboratory of the Francis Delafield Hospital, under the leadership of Dr. Samuel Graff, has pursued studies along a number of different lines. Mr. Kenneth McCarty is using tissue culture techniques as a means of studying intermediary metabolism and as a substrate for virus culture. Drs. Horace B. Gillespie and Morris Engelman have synthesized a number of new triazoles, imidazoles, and quinoxalines. Several of these compounds, among them 4-methoxy-6-nitrobenzimidazole and 4-methoxy-6-nitrobenzotriazole, are effective growth inhibitors of Tetrahymena geleii, a guanine-requiring protozoan, but none of them affected the growth of transplanted mammary carcinoma 755 in mice. About half of the thirty-seven compounds tested in developing frog embryos by Drs. Kathe B. Liedke and Engelman were found to be inhibitory in varying degree. Some of the compounds exhibited selective activity toward particular embryonic stages.

With Dr. Donald Tschudy, Mrs. Ada Graff, and Dr. Margaret Marshall, an investigation has been carried out to trace possible errors of protein metabolism in human leukemia. N^{15}-labeled L-aspartic acid has been used as the tracer in

this study as well as in the demonstration by Drs. Elliott Osserman and Marshall, Mrs. Graff and Miss Dolores P. Lawlor that the protein excreted in one case of multiple myeloma was synthesized with abnormal speed and rapidly excreted by the kidney. A parallel experiment involving the metabolic behavior of parenterally administered adenine in leukemic white cells is being carried out by Dr. Aaron Freedman.

Dr. Engelman, Mrs. Graff, Mrs. F.J. Agate, and Mr. Gerard Zagal have made progress in the search for the substance in the milk of old Storrs-Little mice which increases the incidence of leukemia in C58 mice. This substance appears to be of low molecular weight since it is present in milk ultrafiltrate.

Dr. Victor Ross is conducting an informative chromatographic search for unrecognized or abnormal nucleic acid metabolites in the fluids and tissues of cancerous mice. Drs. Alvan L. Barach, Hylan A. Bickerman, and Gustave Beck completed an experimental study of the results of acclimatization and the effects of partial hypoxia on tissue growth. A decrease in tumor weight, without loss in body weight, is produced in C57 mice bearing Sarcoma 180 or mammary carcinoma 755 by maintaining them for 10 days in a chamber at 9.5 per cent oxygen. The tumors themselves manifested no capacity for increased resistance to hypoxia after repeated exposure to an acclimatization procedure which manifestly protected the host.

Dentistry

Research in the Department of Dentistry of the Francis Delafield Hospital continues to be concerned primarily with the clinical and laboratory evaluation of various types of drug therapy. Some problems of oral pathology have also been investigated. A number of the studies mentioned in last year's report have been brought to completion and appear as literature references in this year's bibliography.

Drs. J.D. Piro, E.E. Sproul, E.V. Zegarelli, and A.H. Kutscher have evaluated a series of new local anesthetic agents (cyclaine, bonocaine A, 2-chloroprocaine, and oracaine) by intra-oral injection into the muco-buccal fold areas and found them to be free from any gross or microscopic deleterious effects. With Drs. L. Seguin, S. Lewis, R. Rankow, and R. Segall, they have completed a cross-relation of the differing sensitivities of 265 pathogenic microorganisms to nine antibiotics according to disc sensitivity tests. With Drs. J. Budowsky and A. Barnett, a study of radiation exposure to the head and abdomen during oral roentgenography has been carried out.

A number of other investigations by various members of the group are in progress or are being prepared for publication. These include the following: studies in humans of the subcutaneous pain threshold with pressure using the Hardy-Wolff-Goodell algesimeter; evaluation of the effect of antihistamines and other drugs on the pain threshold; clinical evaluation of phenaphen as an analgesic agent; continuation of the disc sensitivity study to include the newer and also the older antibiotics in terms of all organisms isolated in the bacteriological laboratory; killing-time studies on Bradosol *in vitro* against *Monilia albicans*; effect of radiation resulting from full mouth x-rays on the cellular elements of the blood ; problems in mass dental extractions in adrenalectomized patients; incidence, etiology, and treatment of cheilosis in debilitated terminal carcinoma patients; histopathology of oral pigmentations and associated intestinal polyps in Jaegerb syndrome; histopathological evidence of pathology following

the injection of local anesthetics alone or in combination with polyvinylpyrrolidone; and evaluation of a reported relationship between the occurrence of torus palatinus and breast or uterine carcinoma.

Gynecology

Research in gynecologic cancer continues to be an important part of the work of the Department. Studies during the current year may be outlined under a number of different headings.

The Steroid Hormones and Cancer

The relationship of steroid hormone metabolism and the development of various types of new growth, especially those of the reproductive organs, continues to be an important field for research in the Department. The center of work in this field is being carried on in the laboratory of Dr. Seymour Lieberman. Investigations of the nature and amount of 17-ketosteroids being excreted by cancer patients both spontaneously and under treatment with chorionic gonadotrophin continues as a major activity. An elaboration of that problem is a study of the role of the liver in determining the nature of the excretion of steroid metabolites in cancer patients following the administration of exogenous C^{14}-labelled steroids. This work under Dr. Lieberman's direction is being carried on particularly by Dr. Samuel Solomon and Mr. Stanley S. Lerner.

Beside the direct clinical studies of the steroids in cancer, the general investigations of steroid hormone biochemistry and physiology being carried on by Dr. Lieberman and his group are relevant. These include the biosynthesis of steroid hormones by means of the perfusion of isolated glands, such as the adrenals, ovaries, testes and placenta with C^{14}-labelled materials; the studies of methods for the synthesis and hydrolysis of steroid glucuronosides and the preparation of steroid-protein complexes and the investigation of their chemical, immunological and biological properties (with Drs. Bernard Erlanger and Sam Biser of the Department of Microbiology and Dr. Frederic J. Agate, Jr. of the Department of Anatomy).

Differentiation in Gynecologic Neoplasms

The program designed to study the cytochemistry and biochemistry of differentiation in gynecologic neoplasms, particularly in the epithelial tumors of

the ovary, has continued along the lines described in the two previous years.

The preliminary steps consist of the collection of all epithelial tumors of the ovary and their classification according to degree of malignancy on the basis of conventional microscopic slides. In an effort to find measurable qualities of tissue that might be correlated with degree of malignancy, each specimen is then subjected to a number of biological, cytochemical or histochemical tests. At the beginning of the work two years ago, some twenty different determinations were selected for investigation. During the last year most of these have been continued, some have been dropped as impractical and a few have been added.

Since the beginning of the study on July 1, 1952, a total of 146 malignant neoplasms have been obtained, consisting of 39 papillary serous tumors of the ovary; 22 papillary pseudomucinous ovarian tumors; 18 endometrial carcinomas; 34 epidermoid carcinomas of the cervix and 33 miscellaneous types.

The original grading has been carried out on the basis of routine hematoxylene-eosin preparations by Dr. Margaret E. Long, and by Dr. Howard C. Taylor, Jr. A preliminary part of the work was concerned with a study of the consistency of grade in individual tumors. A series of clinical cases were collected in which a recurrence or a second tumor developed from a few months to many years after the time of the first operation and it was demonstrated that the grade of the two samples was remarkably constant in spite of the lapse of time. Secondly, some 550 sections from 39 different tumors were studied in order to determine the consistency of grade in different areas of the same neoplasm at the time of their removal. It was again determined that the grade of a papillary serous tumor of the ovary is remarkably consistent throughout its original extent.

Cytochemical studies have been carried on particularly by Dr. Long, based upon sections, samples of ascitic fluid and tissue smears. She has been studying ribonucleic acid, succinoxidase, alkaline phosphatase, glucose and a number

of other substances. The portion of the study already completed concerns the ribonucleic acid of the nucleolus. Her work has demonstrated that there is a steady increase in the number of nucleoli per nucleus and in the average size of the nucleolus per nucleus as the grade of malignancy increases. This observation represents one rather clear-cut cell characteristic which varies with the grade of malignancy.

The study of the RNA of the nucleolus has been reduced to a relatively quantitative technique by the actual counting of the nucleoli and by the measurement of the diameter of the single nucleolus. The study of the desoxyribonucleic acid (DNA) cannot be undertaken by this method. To solve the problem of measuring DNA, Dr. Saul Bader has recently been added to the staff and is undertaking the measurement of this substance by spectrophotometric methods.

The biochemical study of the nucleic acids, in relation to grades of malignancy, is being carried on by Dr. Dudipala V. N. Reddy with a laboratory in the Delafield Hospital. His work has involved the isolation of the nuclei by differential centrifugation and the study of the amounts of each nucleic acid. Based on the study of ten ovarian and nine special tumors, he has found a tendency for the tumors of higher grade of malignancy to show a higher composition of DNA per nucleus than the benign tumors. The ratio of RNA to DNA appears significantly higher in the benign and borderline tumors than in the malignant ones.

Enzymatic studies have also been carried out by Dr. Reddy on samples of these tumors. There was a tendency for the enzyme systems D-amino acid oxidase, cytochrome oxidase and succinoxidase to disappear toward the more malignant end of the tumor series. Dr. Reddy is now working on the rate of uptake of radioactive phosphorus by tumors of different grades of malignancy, but the results in this field are too preliminary to report at present.

Within the general program for the study of differentiation of tumors, Dr. Helena DeRoetth is carrying out certain metabolic studies and is investigating the soluble proteins of tumors. In general, the results show a progressive in-

crease in the rate of oxygen uptake with increasing malignancy, this year's results bearing out in general those obtained in previous years. Work on the incorporation of C^{14}-labelled glucose is in progress but results are still preliminary. The soluble proteins of ovarian tumors are being measured both by the Tiselius and by paper electrophoresis. There is some evidence that the type of protein may vary according to the different degrees of malignancy.

In collaboration with the workers in the College of Physicians and Surgeons, studies with samples of identical tumors are being carried on by Dr. Michael J. Kopac, Professor of Biology at New York University Washington Square College. His group is particularly concerned with ultimate cellular behavior, including tissue culture, micrurgy and special cytologic and cytochemical reactions of single cells.

The work on the degrees of differentiation, or otherwise expressed, upon the grades of malignancy, has proved as complicated as was expected. It does appear at this point that certain differences are appearing which may make it possible more precisely to define those qualities of the cell, chemical and biological, which characterize it as malignant.

Studies on the Transplantation of Human Tumors

A small laboratory has been established under the direction of Dr. Harold M.M. Tovell to study the problems of the heterologous transplantation of human tumors into alien species, namely into weanling rats and hamsters. Twenty-two gynecological tumors have been transplanted, of which two have shown themselves to be serially transplantable. The preliminary work indicates therefore that possibly 90% of human tumors may be transplanted and that 5 to 10% may then be serially transplantable. This is a confirmation of work done elsewhere and appears to lay the ground for a number of important future experiments.

Radiosensitivity

The problem of decision between radiation and surgical treatment in cancer of the cervix continues a vital one. Efforts to solve this by histological

techniques for detecting radio-sensitive cases continues in many institutions throughout the world. The work in this institution is under the clinical direction of Dr. Saul Gusberg in cooperation with Dr. Margaret E. Long in charge of histochemical techniques. The method here, in contrast with that elsewhere, is based upon the effect of small preliminary doses of radiation upon the cancer cell particularly upon the nucleic acid components of the nucleolus. On account of the time required to collect sufficient cases and to observe outcomes, it is evident that this work will require many years to complete.

The Ovary in Cancer of the Cervix

Work previously reported attempting to measure quantitatively the amounts of functioning ovarian tissue and to relate these, if possible, to the development of neoplasms in the gynecologic organs continues. Much progress has been made in the actual number of ovaries studied, but the requirements of statistical significance indicate that several more years may be required to arrive at either a positive or negative conclusion.

Clinical and pathological studies of gynecologic cancer continue in many directions. Dr. Iandrum B. Shettles has published a paper on chorionepithelioma of the uterus and Dr. Ferdinand H. Flick and Dr. Richard S. Banfield, Jr. have reviewed the pathology and end results in cases of granulosa cell tumor and thecoma of the ovary. Dr. James A. Corscaden has continued his interest in this subject and has published papers on the "Interstitial Radium Treatment of Cancer of the Cervix" and on "The Management of Carcinoma of the Corpus", respectively. He has completed the revision of the second edition of his book "Gynecologic Cancer".

CLINICAL INVESTIGATION IN GYNECOLOGICAL CANCER

The plan set up three years ago for a cooperative approach by the divisions of radiotherapy and gynecological surgery continues. The original plan called for an alternation of cases, wherever justifiable, between a plan of therapy which was predominantly by radiation and one that was primarily surgical. This plan

has been continued for cancer of several types, but has been abandoned in some situations where one method has appeared rather definitely more promising than another.

Stages I and II of cancer of the cervix are being treated by two methods. One group is being referred for an immediate operation consisting of a radical hysterectomy followed by lymph node dissection. The other group is receiving full primary radiation therapy and then, after a three month interval, subjected to an operation for removal of the iliac lymph nodes through an extra-peritoneal approach. The two series should eventually give interesting data on the relative value of surgery and radiation as the treatment of choice and should rather soon lead to conclusions as to the effectiveness of x-ray in diminishing the frequency with which iliac node metastases are found. In the more advanced cases, immediate surgery has been at least temporarily abandoned and all cases receive the possible benefits of radiation before an exenteration operation is considered.

Progress in the last year should also be noted in the improvement of the follow-up system, which has been brought about by Dr. David B. Moore. The systems in the gynecological clinics of the Presbyter ian and of the Delafield Hospitals have been made similar and set up under one set of workers. This should add greatly to the accuracy and completeness of follow-up data being obtained.

Medicine

The widely varied problems of disseminated neoplastic disease provido fruitful areas for investigation. During the past year studies have been carried out at the laboratory and clinical levels on such diverse subjects as mechanisms of anti-tumor drug action, experimental chemotherapy of animal and human tumors, protein and electrolyte metabolism in human cancer patients, the anemia of malignancy and tobacco tar carcinogenesis. The research has been carried out in laboratories at the College of Physicians and Surgeons, and the laboratories and medical wards of the Francis Delafield Hospital. Many of the investigations have been cooperative ventures among various members of the staff. This has been particularly true of the clinical cancer chemotherapy studies. The professional staff includes the following:

Jonathan Bishop, M.D. *	Paul Marks, M.D. *
Daniel Choy, M.D.	Jan Mijnssen, M.D.
Charles Crandall, M.D.	Elliott Osserman, M.D.
Jack Davidson, M.D.	Helen Ranney, M.D.
Philip Feigelson, Ph.D.	Laszlo Reiner, M.D., Ph.D.
Rainer Fried, Ph.D.	Martha Safier, M.D. *
Alfred Gellhorn, M.D.	Helga Schwarz *
Erich Hirschberg, Ph.D.	Tae Sup Song, M.D. *
George Hyman, M.D.	Sedat Yorukoglu, M.D. *

* = Resident House Officers

In the following paragraphs work in progress as well as that completed during the year will be briefly summarized.

Dr. Jack D. Davidson was joined this past year by Dr. Philip Feigelson in a continuation of studies upon the mechanisms of action of anti-tumor drugs. Up to the present attention has been focused upon the effects of these compounds on nucleic acid metabolism. The techniques employed have involved radioactive tracers and enzyme studies.

The tracer work has used P^{32} orthophophate, C^{14}-formate, and adenine-8-C^{14}. These have been employed to investigate the influence of drugs upon nucleic acid biosynthesis in solid and ascites tumors in mice. 8-Azaguanine caused marked inhibition of the incorporation of the adenine skeleton into both adenine and guanine of RNA and DNA. This was accompanied by depression of the specific activity of the free hypoxanthine in the tissue. More extensive studies of the acid-soluble purine intermediates have recently been started. These have been facilitated by the development of methods for the liquid scintillation counting of C^{14} in purines. Radiophosphate studies were completed for 27 compounds in the Cancer Chemotherapy Screening study of the American Cancer Society and the results have been published.

Enzyme studies to determine the precise biochemical locus at which antitumor drugs exert their effects were initiated by examining in vitro effects of these drugs on certain isolated enzyme systems. It was found that adenosine deaminase, an enzyme important in the catabolism and possibly the anabolism of the purine ribosides, was profoundly inhibited by 8-azaguanine in both homogenates of tumor 755 and in purified enzyme preparations. Pharmacological concentrations of 8-azaguanine induced inhibition and a concentration of 0.001 M 8-azaguanine completely inactivated a purified adenosine deaminase preparation. The precise mechanism by which the 8-azaguanine inhibits this enzyme is currently under investigation.

Studies on the degree to which mammalian tissues may form adaptive enzymes are being continued. Recent findings indicated that subsequent to prolonged parenteral administration of xanthine C57 mice developed elevated liver xanthine oxidase and adenosine deaminase levels. This suggests that both adaptive enzyme and sequential adaptive enzyme formation may occur. The precise mechanism underlying this phenomenon is being further explored in an attempt to ascertain whether this is truly induced enzyme formation or whether these changes reflect a non-specific response possibly hormone mediated.

Dr. Elliott Osserman has continued studies of the serum and urine proteins in neoplastic diseases, particularly multiple myeloma. The introduction of the technique of filter paper electrophoresis as the routine method for serum protein determinations on ward and clinic patients of the Delafield Hospital, has afforded a unique opportunity for a comprehensive survey of the qualitative and quantitative changes of these blood constituents associated with various tumors. From this survey, the series of cases of multiple myeloma has been extended to 75. This has provided a much broader perspective of the clinical and biochemical variants of this "disease group". To date, the serum and/or the urine electrophoretic patterns have demonstrated the presence of a unique and characteristic abnormality in all but three of these 75 cases. These particular 3 cases did, however, exhibit one major phase of the protein derangement of myeloma, i.e. the striking deficit in production of normal gamma globulin.

Physico-chemical studies of the myeloma globulins are in progress. Of particular interest has been the observation of a systematic difference in carbohydrate content between the serum and urine myeloma globulins when both are present in a particular case. It has been found that the serum constituents react strongly with periodic acid-Schiff (PAS) treatment, whereas the corresponding urine constituents are PAS-negative. Further exploration of this carbohydrate aspect has been undertaken in cooperation with Dr. Zacharias Dische in an attempt to identify the carbohydrate bound to the abnormal myeloma globulins.

In cooperation with Dr. Samuel Graff of the Biochemistry Division, a study has been carried out on the dynamics of incorporation and turnover of N^{15}-labelled L-aspartic acid in the abnormal serum and urine proteins of a case of multiple myeloma. The results of this experiment indicate that these two proteins have quite dissimilar turnover rates, and are apparently independently elaborated.

Dr. George Hyman has pursued studies on the mechanism of anemia in cancer. The results of initial investigations on the problem of the unexplained anemia

which appears with advancing malignant neoplastic disease disclosed a hemolytic factor present in patients with carcinoma as well as with lymphoma and leukemia. It was found that routine studies used to demonstrate increased hemolysis such as reticulocytosis, increase in indirect serum bilirubin, increase in fecal urobilinogen and urinary urobilinogen, and increases in red cell fragility did not appear either consistently or to any degree in patients with anemia. Using the Ashby technique of differential red cell agglutination, it was found that the life span of erythrocytes from normal donors was considerably shortened when transfused into patients with malignant disease. These studies were confirmed in other experiments in which the donor cells were labelled with radioactive chromium 51. Attention was then turned toward the status of marrow activity in anemic cancer patients. Using radioactive iron 59 in association with marrow aspiration studies, it was found that marrow activity and rates of erythropoiesis were apparently normal or increased in the majority of these patients, although the increase was not more than one or twofold at most. This demonstrated that marrow replacement is not a major factor in the genesis of the anemia in malignant neoplastic disease.

A sidelight of this study indicated the ease and feasibility of iliac marrow aspiration in the documentation of metastatic neoplastic disease involving bone marrow, in that 10% of 650 cases selected at random, on the Medical Service at Francis Delafield Hospital, yielded specimens of marrow that revealed tumor cells in clumps. This method is being applied to the preoperative survey of certain patients with apparently operable carcinoma in an attempt to avoid unnecessary surgery.

The latest investigation from this laboratory has concerned itself with a possible intrinsic defect in the red blood corpuscles of patients with neoplastic disease. In a study of 56 volunteers from Sing Sing prison and 26 patients from Francis Delafield Hospital it was found that whereas normal red cells transfused into patients had a considerably shortened life span, the red cells from the

patient when transfused into normal volunteers had a normal or almost normal life span. This seemed to document the absence of an erythrocytic factor and gave additional emphasis to the presence of a hemolytic plasma factor in patients with this condition. Studies are under way to elucidate the nature of this factor, and to determine the effect of anti-tumor therapy on it.

Studies were made on the effect of total body radiation, nitrogen mustard and triethylenemelamine on the hematopoietic system of cancer patients. It was found that these patients were able to tolerate larger doses of total body radiation without significant depression of rates of erythropoiesis than had ever been considered possible, up to a total of 150 r. It was also found that determinations of rates of erythropoiesis with Fe^{59} were sensitive enough to detect small changes produced by low doses of nitrogen mustard and triethylenemelamine.

Dr. George Hyman has cooperated with Dr. Algernon Reese of the Eye Institute in studies of children with bilateral retinoblastoma. Combination therapy has been evaluated using triethylenemelamine orally, intramuscularly, and even via the intra-arterial route together with orbital radiotherapy. This technique seemed to yield more satisfactory results in the treatment of retinoblastoma than had previously been obtained with radiotherapy alone. This was shown by an increase in the apparent cure rate with useful vision and by the decrease in the late sequelae of retinal hemorrhage.

Dr. Paul Marks joined the Medical Staff this year and has organized the Metabolic Ward and Laboratory for clinical research on disturbances of metabolism in neoplastic disease. Investigations are in progress on changes in electrolyte, mineral, nitrogen and carbohydrate metabolism associated with malignant tumors, but results are still too preliminary to report. Dr. Jonathan Bishop is collaborating in these experiments.

Drs. Daniel Choy, Jan Mijnssen and Alfred Gellhorn have devised a model clinical experiment to evaluate the anti-ematic activity of chlorpromazine. In this project the placebo and the active drug are packaged in coded containers

and neither the patient nor the evaluating physician knows the code. This "double blind" test provides better opportunity to avoid any bias in noting the results of any experiment. It is anticipated that the observations will be completed within the current year.

Dr. Sedat Yorukoglu has studied the phenomenon of hypermetabolism in patients with disseminated neoplastic disease. In this investigation the basal metabolic rate, radioactive iodine uptake and the protein bound iodine has been correlated. Similar observations have been made in thyrotoxic subjects. It was found that many cancer patients had elevated basal metabolic rates but normal uptake of radioiodine by the thyroid and normal protein bound iodine concentrations in the blood. In thyrotoxic patients these values are all uniformly increased and are decreased by the administration of cortisone or ACTH. The adrenal cortical steroids do not modify the hypermetabolism associated with cancer.

Dr. Charles Crandall and Dr. Sedat Yorukoglu have been cooperating with the Urological Service in an evaluation of bilateral adrenalectomy in breast cancer. An attempt has been made to study all possible parameters preoperatively with the objective of determining those criteria which may be most useful in selecting appropriate cases for the procedure. The results to date do not as yet suggest which criterion or which criteria are helpful. Of particular interest in the investigation has been the frequency of hypercalcemia and the value of intravenous ethylenediaminetetraacetic acid in the treatment of this serious complication.

Dr. Helga Schwarz has carried out a careful clinical study of a patient with Wegener's necrotizing granulomatosis. This unusual 13 year old patient has had a fascinating course in which the life saving action of cortisone and meticorten have been dramatically demonstrated.

Dr. Alfred Gellhorn and Dr. Erich Hirschberg served as Chairman and Executive Secretary in an inter-institutional investigation of cancer chemo-

therapy screening methodology. The study, carried out by 24 investigators in eight institutions, consisted of the evaluation of 74 biological systems including experimental animal tumors, microorganisms, systems involving differentiation and development, and biochemical synthetic reactions as possible guides for the anti-tumor activity of a selected series of twenty-seven compounds. Drs. Gellhorn and Hirschberg were responsible for four of the seventy-four individual systems (three transplanted tumors and the slime mold Dictyostelium discoideum) and were active in the overall conduct of the study; collection, analysis, and correlation of the data; and editing of the final report which was published as a supplement to Cancer Research.

The evaluation confirmed and extended previous observations on the variability of response among experimental neoplasms to chemotherapeutic agents; no single tumor was able to select all useful agents and a spectrum of tumors was shown to provide a greatly improved screening system. None of the non-tumor systems included in this study were able to replace a tumor system as a screening tool for carcinostatic agents. Correlation of the data obtained in experimental tumors with information available on the clinical effectiveness of a number of these agents pointed up the incompleteness of present clinical knowledge on the response of human neoplasms to many compounds which are active against experimental neoplasms.

The investigation of the response of brain tumors in vitro and in vivo to a variety of chemotherapeutic agents continues in collaboration with Dr. Margaret Murray and Miss Ruth Fricker. Mouse Glioma 26, a methylcholanthrene induced transplantable brain tumor, is the chief source for these studies. In addition to the purine analogs 6-mercaptopurine, thioguanine, and 8-azaguanine, several sulfonoxyalkanes, benzimidazoles, and acridines have been tested. In general, the tissue cultures of this tumor are more easily inhibited than the cells in vivo, since they can usually be exposed to higher concentrations of the agent under investigation. Occasionally, however, as with 6-mercapto-

purine, effective carcinostasis in the mouse is accompanied by failure to affect the tumor in tissue culture; the reasons for this discrepancy are under investigation.

This type of study has now been extended into another direction. It has been mentioned in earlier reports that samples of human glioblastoma multiforme can be grown in tissue culture and that this system can be used for the evaluation of carcinostatic agents. It has also been found that tumors obtained from different patients may differ in their response to these agents for a variety of reasons, including distribution of various glial cell types in the sample and growth rate of the tumor. With a view toward possible clinical applicability of tissue culture results, a series of patients with these malignant brain tumors have been given chemotherapeutic agents selected on the basis of the response of their particular tumor in vitro following removal at operation. Since survival time of these patients is the most adequate criterion of therapeutic success, it is not yet possible to assess the value of this procedure; however, the clinical trial of agents selected on this basis has been sufficiently encouraging to warrant further attempts to correlate in vivo and in vitro effects.

In the tissue culture trials with these samples of human tumors, the most interesting group of compounds to date are a series of acridine derivatives, which appear to be at least a hundred times more effective than 8-azaguanine in vitro. These compounds are under active investigation at the present time.

Dr. Erich Hirschberg has continued studies on biochemical mechanisms of differentiation using the slime mold Dictyostelium discoideum. It has been found that a number of 2,4-diaminopyrimidines and dihydrotriazines inhibit important steps in differentiation. Reversal experiments, now in progress, have demonstrated that folic acid and uracil effectively counter the action of these antibacterial, antimalarial compounds in this system.

Dr. Laszlo Reiner and Dr. Alfred Gellhorn have investigated the intracellular localization of quinacrine in normal and neoplastic cells. This project was undertaken because of the suggestion made by others that there was a hitherto undescribed intranuclear component with a high affinity for quinacrine. In a series of in vitro and in vivo experiments in which whole cells and separate components were exposed to varying concentrations of the compound, the conclusion was reached that quinacrine was not specifically bound by any cell component and that the cellular distribution could be accounted for on a physico-chemical basis. The studies here mentioned led to the trial of acridines against brain tumors as described in an earlier paragraph.

Many of the members of the group have participated in the clinical evaluation of cancer chemotherapeutic agents. The following have been particularly responsible for this activity: Drs. Charles Crandall, George Hyman, Sedat Yorukoglu, Helen Ranney and Alfred Gellhorn.

The drugs which have been studied during the past year are: Nonane (1,4-dimethylsulfonoxynonane), CB 1348 (p-carboxypropyl)phenyl bis beta chloroethylamine), quinacrine, Meticorten, 6-mercaptopurine and Sarkomycin. The results of these therapeutic trials have been collected for the Cancer Chemotherapy Center of the National Cancer Institute. The Table summarizes the overall results.

Drug	Disease	No. of Pts.	Results
6-Mercaptopurine	Acute leukemia	35	17/35 improved
	Lymphomas	20	4/20 improved
	Multiple myeloma	15	2/15 improved
	Carcinomas	17	No effect
Nonane	Multiple myeloma	3	No effect
	Ca of Cervix	1	No effect
	Ca of thyroid	1	No effect
	Bronchogenic Ca	3	No effect
	Ca of breast	4	No effect
	Ca of intestine	4	No effect
	Leiomyosarcoma	2	No effect
	Glioblastoma	3	No effect
Thio TEPA	Lymphosarcoma	10	4/10 improved
	Hodgkin's disease	2	No effect
	Ca of ovary	4	2/4 improved
TEM (triethylene melamine)	Ca of ovary	10	3/10 improved
CB 1348	Chronic lymphatic leukemia	11	4/11 improved
	Hodgkin's disease	2	1/2 improved
	Lymphosarcoma	2	1/2 improved
Meticorten	Acute leukemia	8	3/8 improved
	Lymphosarcoma	9	8/9 improved
Quinacrine	Glioblastoma	12	2/12 improved
Sarkomycin	Bronchogenic Ca	4	No effect
	Ca of uterus	5	No effect

Neurological Surgery

Three broad avenues of investigation are being pursued by the Department of Neurological Surgery at the Francis Delafield Hospital. These are 1) development of methods of relief of pain for patients with cancer, 2) collaborative efforts with members of the Departments of Medicine, Surgery and Radiotherapy to influence, biochemically, malignant tumors of the brain, and 3) the acquisition of knowledge of function of the nervous system which may be obtained by careful observation of neurological changes effected during neurosurgical procedures for relief of pain.

Dr. J. Lawrence Pool and Dr. Kemp Clark have developed a technique for delivering small, carefully regulated, electrical stimuli to the depths of the frontal lobes for relief of pain. They have collaborated with Dr. M. Lombardo and Dr. Perry Hudson in a study of blood and urine steroid changes produced by the frontal lobe stimulation.

Drs. Thomas Bridges and Clark are investigating a method of "quantitatively" producing frontal lobotomy by injection of warm, normal saline into the frontal lobe through small trephinations. It is possible to produce sufficient interruption of fiber pathways to relieve pain, without producing such side-effects as extreme lethargy and indifference and is of greatest value in making terminal patients comfortable. There have been no undesirable side-effects from such injections.

Drs. J.L. Pool, J. Ransohoff and T. Bridges are collaborating with Dr. Alfred Gellhorn in studies of the effects of oral "antimetabolite" drugs upon malignant tumors, some of which receive concurrent radiotherapy as an additional factor.

Drs. Clark and Bridges have developed a method of continuous intracarotid administration of water-soluble antimetabolite drugs, to produce a higher concentration of drug in a brain tumor.

Operative procedures for relief of pain have offered an opportunity to study physiological processes of the human nervous system, inasmuch as the nervous system

is usually normal in these patients with visceral carcinoma. A study of the effect of stimulation of dorsal and ventral nerve roots upon vasomotor responses in the digit has been completed by Drs. Melvin Yahr and Thomas Bridges.

Observations of the reactivity of cerebral vessels have been made by Drs. Yahr, Clark and Bridges. It has been found that cerebral vessels are maintained in a state of moderate constriction, that they react to external stimulation and that these reactions are reduced by interruption of the cervico-thoracic sympathetic outflow.

Pathology

Investigations begun the previous year by Dr. Silvio Fiala, Dr. Edith Sproul, Mr. Martin Blutinger and Mrs. Anna Fiala were enlarged and the report of the findings accepted for publication. This was a study of the cytoplasmic particulates of normal and tumor tissue by high speed centrifugation. Fractions were checked in the electron microscope with the aid of Mr. Kenneth McCarty. The basophilia of the various subcellular particles was demonstrated by the use of pyronine and correlated quantitatively with their RNA content. This histologic basophilia was related to the microsomal or chromidial fractions. Quantitative differences existing between tumor and normal cells and between rapidly and slowly growing tumors in content of heavy and light particles were found. Tumor tissue is characterized by scarcity of large granules (mitochondria and heavy chromidia), reduction in amount of small chromidia (microsomes) and predominance of basophilia in non-sedimented smallest nucleoprotein granules. Tumors are thus distinguished from pancreas in which mitochondria are also scarce but heavy and light chromidia abundant.

Studies on the interdependence of cytoplasmic particulates by Drs. Fiala and Sproul and Mrs. Fiala were continued. In one aspect of the work, the effect of ACTH on subcellular fractions of the adrenal gland was investigated to determine what structural changes accompany the induced hyperfunction. The findings concerning this tropic hormone bear on some of the fundamental problems of normal and pathologic growth. It was demonstrated that ACTH promptly provokes an increase in adrenal cytoplasmic RNA and growth of the basophilic chromidial system independent of cell division. The delayed increase in mitochondria seemed to be dependent upon the chromidial system.

Another avenue of study by the same investigators had to do with the localization of binding of the cancerogen 3,4-benzpyrene to the proteins of the RNA-rich chromidial system of mouse epidermis rather than to the mitochondria or large chromidial granules. Benzpyrene was found to have no effect on respira-

tion of mouse epidermis slices although its presence in the skin could be demonstrated. The evidence suggests that the cancerogen may act by diverting protein synthesis from the formation of differentiated respiratory mitochondria to a hyperproduction of a simpler glycolytic system.

Dr. Sproul and Mr. Blutinger have been comparing by histochemical technics the intercellular cement substance, stromal ground substance and vascular basement membranes of normal, benign and malignant human tissues. Conclusions cannot be drawn until a variety of lesions are examined. Meanwhile changes in epidermis and stroma were observed during the course of methylcholanthrene cancerogenesis in mouse skin. While the chemical nature of the alterations cannot be defined by colloidal iron or metachromatic staining, the findings suggest depolymerization or hydrolysis of the acid mucopolysaccharides before characteristics of malignant cells appear in the epithelium. This work is continuing in expanded form.

Dr. Gerald Fine with Dr. Purdy Stout has been reviewing the osteogenic sarcoma of the soft tissues accumulated in the Presbyterian Hospital and in the literature in an attempt to define their behavior and establish criteria for differentiating them from atypical forms of myositis ossificans.

Dr. Fine has also been collaborating with Dr. I. Waeserman of the Department of Surgery in studying the histologic and metabolic changes resulting from removal of segments of small bowel and (or) by-passing the ileocecal valve.

Dr. Irene Roeckel with the assistance of Mrs. Lucile Seguin began an attempt to culture whole pituitary gland in anticipation of the performance of human hypophysectomies. Lucite chambers after Trowell were constructed. Difficulties in sterilization sufficient for long term organ culture required alteration of the recommended procedure. Proper media were investigated by culturing the anterior pituitary gland removed at autopsy. No contamination was observed in cultures carried for four weeks. Growth stimulation was minimal.

Dr. Sproul has been assisting Dr. Kutscher of the Department of Dentistry

in studying the tissues of patients with Jaeger's syndrome. A report of familial hyperkeratosis of the oral mucous membrane was also prepared by these collaborators.

Radiological Research

In the Radiological Research Laboratories, under the direction of Dr. G. Failla, emphasis has been placed on radiological physics and radiobiology.

Radiological Physics

A number of calibrations involving tissue equivalent ionization chambers have been performed including a comparison with a different type of neutron dosimeter. Experimental measurements of the LET (linear energy transfer) distribution of 3 neutron sources have been carried out and the results compared with theory. In addition, further work on the characteristics of the LET measuring device was performed.

A special ionization chamber has been constructed for the separate assessment of various radiations incident upon borated tissue.

Beta ray dosimetry utilizing chambers and internal gas multiplication is being explored as a specific example of the general application of dosimetry at collecting voltages in excess of saturation.

Data have been obtained on radiation-induced leakage in the dielectric of ionization chambers having small volumes.

Improvement in source preparation technique makes possible the use of the Symmetrical Electrodes 2π Counter with lower energy beta-emitting radioisotopes by reducing selfabsorption. P-32 disintegration rate comparison with Argonne National Laboratories gave satisfactory agreement.

Further experiments on the determination of "W" have been carried out. The stopping power, relative to air, of several gases has been calculated from Bethe-Bloch theory and the results are in very close agreement with the experimental values of Hersh reported last year.

A new method of determining the number of disintegrations per second for K capture isotopes is described. It has been tested with the gamma rays of Au-198.

Experimental data have been obtained which indicate that the mere attainment of a plateau on a saturation curve is not sufficient proof that all the ions pro-

duced in an ionization chamber are being collected. This may help explain energy dependence of "W" for alpha particles.

Investigation of the tissue dose produced by radioisotopes when highly localized in small regions by means of "wall-less" ionization chambers has continued. Some difficulties encountered in this work are described and the design of a new ionization chamber to aid in this investigation is outlined.

Depth dose determinations have been made on several plane, thin sources of beta ray emitters of uniform surface density. The results of calibration of many commercial survey instruments using these determinations are given.

A method for simulating x-ray depth dose curves by light absorption and scattering in a water suspension of gum gamboge and a dye is described. It has been found that individual depth dose curves can be reproduced accurately enough for practical purposes.

Radiobiology

Dr. Roberts Rugh and his associates have conducted studies on the relation of sex, estrus cycle, and hydration to radiosensitivity in the mouse and have shown that female mice are more resistant than males to an LD/50/30 day exposure to x-rays, and females in estrus are about twice as radiosensitive as those in diestrus.

The protection of fetuses from normally lethal doses of radiation by injection of cysteinamine into the pregnant mouse has been reported.

Observation of the effect of radiation on the eyes of fetuses x-rayed on gestation day 12.5 or 13.5 indicate that the fetal eye exhibits remarkable powers of repair and regeneration aided by the radioresistant primitive neurectoderm.

A comparative study of the early cytological radiation effects on the lens epithelium of mice, rats, rabbits, dogs, cats, and rhesus monkeys has been undertaken. Some results suggest an association between the proliferative activity of the lens epithelium in individual species and its radiosensitivity. In con-

junction with this study, the influence of x-radiation on the composition and metabolism of the lens capsule and the lens itself is being investigated.

A study of post-irradiation protection of rabbits by injection of "splenic" plasma is described. The splenic plasma is plasma derived from blood taken directly from the splenic circulation of normal rabbits. Injection of this plasma into irradiated rabbits appears to influence favorably their survival in comparison with that of control rabbits receiving total body irradiation only.

The inactivating effects of x- and ultraviolet radiations on bacteriophage T_1 appear to be independent when the two types of radiation are administered consecutively. Similarly, the effects of heat and ultraviolet are found to be independent when administered consecutively but only if the phage is in aqueous suspension during treatments. If the phage is exposed to UV in the dry state, and subsequently heated while still dry or in aqueous suspension, its sensitivity to heat is increased.

Exposure of E. coli B or B/R to a wavelength band from 3200 Å to 4600 Å produces a very slow reduction in colony formation but a much more rapid reduction in the ability to produce phage T_1. T_1 phage surviving ultraviolet radiation is also not reproduced by previously illuminated bacteria. Work is in progress to determine whether the effects of the illumination can be reversed.

Radioisotope Laboratory

The radioisotope laboratory, under the supervision of Dr. Edith H. Quimby, operates as a receiving, standardizing, and dispensing center for all isotopes used clinically in the Presbyterian Hospital. In addition, the laboratory personnel assist in setting up various isotope research programs throughout the institution.

Therapy with radioactive isotopes

Radioactive iodine continues to be used in treatment of some thyroid cancers as described in last year's report. There are no new developments to report in this program under the leadership of Dr. Virginia Frantz, nor in the use of radio-

active phosphorus in leukemia and polycythemia, under Dr. Harold Jacox and Dr. Morton Kligerman. The use of radioactive colloidal gold in treatment of pleural effusion or ascites due to carcinomatosis, with Dr. Kligerman and Dr. David Habif, has afforded some remarkable instances of palliation. The series of treated patients is continually increasing. No new therapeutic procedures have been developed during the year.

Diagnostic and tracer studies with radioactive isotopes

The routine or service part of the isotope laboratory continues to function as in previous years. Radioactive iodine, both as iodide and labelled human serum albumin, radioactive phosphorus, sodium, and potassium are received, standardized and dispensed to various staff members engaged in research projects. Those applicable to cancer will be reported by the responsible investigators.

Radioautographic studies are regularly made on all suspected thyroid cancers at the time of biopsy or surgery. A survey for potential metastases is made at request of the thyroid cancer group; this involves use of a relatively large test dose and point-to-point checking by means of a scintillation counter.

The search for a satisfactory technique for localization of brain tumors with the aid of radioactive isotopes has been going on for some time in several institutions. Success depends upon higher uptake of radioactive material in the tumor than in surrounding tissues, and in satisfactory collimation of the radiation from the tumor, to minimize the direct and scattered radiation from the rest of the head. A project here has been set up with Dr. Juan Taveras and Dr. Edward Schlesinger of the Neurological Institute and Dr. Harold Rossi and Dr. Quimby of Radiological Research. Apparatus has been developed which appears to give promise of better results than methods previously tried; clinical work is about to be started.

Radium and x-ray physics

The work commenced last year on dosage calculation for horizontal rotation therapy was completed and has been published. A simpler and more direct method

Radiology

Presbyterian Hospital

Dr. Harold Jacox has continued studies on the results of radiotherapy in carcinomas of the breast, lung and uterus.

Dr. Russell Wigh and Dr. Ezekiel Freed have conducted a radiodiagnostic study on carcinoma of the esophagus in which greater precision in delineating the extent of the disease has been attempted. In a number of cases the wall of the esophagus can be visualized as a soft tissue shadow separate from the barium column. In carcinoma of the esophagus, in addition to the filling defect, a thickening of the esophageal wall may be appreciated in the x-ray films. This represents submucosal infiltration which always extends well beyond the apparent mucosal tumor. The observations are helpful in deciding the size of portal necessary for radiotherapy or as a guide to the surgeon of the extent of esophageal resection required.

Drs. Russell Wigh and Nora duV. Tapley have investigated the radiological characteristics which distinguish primary and secondary tumors of the colon. The project was stimulated by the observation of several patients with breast cancer who developed intestinal symptoms and radiologic evidences of bowel tumor. At laparotomy these were found to be metastatic deposits rather than another primary tumor. In the study to date, the smoothness of the tumor mass and the presence of intact mucosa suggest a metastatic intestinal cancer. The anatomical localization of such tumors is in the submucosa and the metastatic pathway is hematogenous.

Drs. John Fertig, Edith Quimby, M. Kligerman and Samuel Madell have conducted a carefully controlled study of the accuracy of I^{131}-labelled albumin for the diagnosis of intrahepatic metastases. It had been reported by others that tagged albumin localized in liver metastases and that the gamma rays from the radioiodine could be accurately detected through the abdominal wall. The investigations in this laboratory, using an accurate directional counter, failed to

confirm the previous reports. No significant difference in the radioactivity of normal liver and liver metastases could be detected.

Dr. Morton Kligerman has initiated studies with Dr. Daniel Shapiro on the effect of radiation and cytotoxins on tumors in mice. The results to date are too preliminary for discussion.

Drs. Juan Taveras, Edward Schlesinger, Harald Rossi and Edith Quimby are attempting to develop improved techniques in radioactive isotopic localization of brain tumors.

Francis Delafield Hospital

A. Radiotherapy

Drs. Maurice Lenz and Ruth Guttmann, together with their associates, have emphasized studies to standardize the tumor dosage needed for clinical restraint of various regional cancers by the use of 2 MeV x-rays and Cobalt^{60} radiation. Inherent radiosensitivity characteristic of the cancer under treatment, its superficial extent and invasiveness, as well as regional lymph node involvement, are the chief factors which influence the outcome of supervoltage radiotherapy, just as with more conventional irradiation. Tumor doses of 5000 - 6000 r in five to six weeks have been followed by growth restraint in a few deep-seated cancers of limited extent, not usually controlled by less penetrating irradiation, but also by failure of the treatment in many other more extensive cancers. The irradiated normal tissues seem to tolerate these high tumor doses better when given with supervoltage than with more conventional type of x-ray in the 200 or 250 K.V. range. Thus, there have been arrested some cancers of the base of the tongue, maxillary antrum, cancer of the larynx with fixed vocal cords, cancer of the breast with internal mammary node metastases, recurring multiple papillary carcinomas of the urinary bladder, and some cancers of the cervix uteri.

Of 14 carcinomas of the lung thus treated and examined post mortem by Dr. Edith Sproul, three failed to show any evidence of persisting cancer in the

treated area. Marked palliation of cough, reduction of expectoration, bleeding and pain and perhaps some prolongation of survival was obtained in the majority of 100 carcinomas of the lung investigated by Dr. Guttmann.

A special study was made of cancer of the esophagus by Drs. Thomas O'Grady and Lenz. Review of the literature and of 40 personal cases treated on the 2 MeV and Cobalt^{60} equipment suggests that cancer of the esophagus primarily spreads in a vertical direction along the submucosal lymphatics, and is often much more extensive than it would appear from esophagoscopy or x-ray examination. Metastasis to lymph nodes draining the primary site is the rule, especially in locally invasive and extensive cases. Cancer of the cervical esophagus in most instances metastasizes to intrathoracic lymph nodes and in only a small percentage to cervical lymph nodes. Those arising in the thoracic esophagus involve not only peri-esophageal and bronchotracheal lymph nodes, but in a goodly percentage also the sub-diaphragmatic lymph nodes. Diagnosis is usually made only after silent involvement of these various lymph nodes has already taken place. Thus, any improvement in treatment of the primary tumor in the esophagus by surgery or radiotherapy is not likely materially to change the poor survival statistics after these purely local methods of treatment. Radical excision or irradiation of all lymph node involvement is practical only if this involvement is of limited extent, i.e. when the primary lesion is small and superficial and has as yet not given rise to extensive, inaccessible and clinically unrecognizable regional lymph node metastases. Improvement of local surgery or radiotherapy is therefore less likely to increase survival than earlier diagnosis. This may be facilitated by the more general and earlier use of biopsy via the esophagoscope and of x-ray study of the mucosal pattern of the esophagus, whenever there is the slightest suspicion of esophageal cancer. The tendency to silent vertical extension and regional lymph node involvement should be taken into consideration in planning either surgery or radiotherapy in the individual patient.

B. Radiodiagnosis

Dr. Arnold Bachman and his associates Drs. Doris Bate, Rose Louie and Jose Guzman have continued an active investigative program on the many problems of radiological diagnosis in neoplastic disease. During the past year work was completed on a radiographic-pathologic study of skeletal metastases. In this valuable contribution it was demonstrated that all intertrabecular and many osteoblastic metastases failed to be identified radiologically. The greatest accuracy in x-ray diagnosis occurred when osteolytic tumors were present.

Dr. Bachman and his associates completed a study of the radiological characteristics of colon cancer which extends into the stomach. A project of bilateral femoral venography was concluded with the demonstration of the feasibility and usefulness of visualization of the pelvic veins and plexuses.

During the present year a number of new studies have been initiated. Drs. Louie and Guzman are investigating the value of duodenal displacement as a criterion of retroperitoneal tumors as contrasted with hepatomegaly. With the wealth of material which comes through the x-ray department it has been possible to show that this sign is of little value in the differential diagnosis.

Dr. Doris Bate has started a systematic study of the frequency, time of appearance, radiographic characteristics, differential diagnosis and duration of pulmonary fibrosis following radiotherapy of breast cancer. This is an important and practical issue since large doses and many sites are being radiated in the treatment of this tumor.

Dr. Bachman, together with Dr. Charles Crandall of the Medical Service, is studying the radiographic usefulness and toxicity of large doses of cholografin in intravenous cholangiography. Although doses up to three times the usual dose have been employed (120 cc rather than 40 cc), significantly superior visualization of the biliary tree has not been achieved.

C. Radiobiology

Dr. Anna Goldfeder has investigated the effects of lethal x-radiation on cellular biochemical constituents. In mice receiving total body x-ray it was found that alkaline phosphatase concentrations in the duodenum, liver, spleen, kidneys and mammary glands were not affected whereas the adenine level in the liver was significantly reduced as compared to control animals.

In a brilliantly conceived and executed series of exporiments, Dr. Goldfeder studied the question of whether or not it is possible to induce immunity in homozygous hosts to endogenous tumors by irradiation of these tumors. Under the experimental conditions of her experiments, Dr. Goldfeder was unable to demonstrate that palliative or curative radiotherapy made the tumor antigenic for its isogenetic host. These experiments are of particular interest at the present time because of the recurrent interest in tumor immunology. Dr. Goldfeder's studies point out the need for careful and critical protocols in this area.

During the current year Dr. Goldfeder observed a spontaneous transformation of a carcinoma to a sarcoma in a tumor of DBA mice on serial transplantation. The suddenness of the change indicated that a mutation had occurred rather than that cell selection played a dominant role.

Surgery

During the past year, the electrogastrogram laboratory under the direction of Dr. Edmund N. Goodman has added the services of Dr. Henry Colcher of the Department of Medicine and Dr. Melvin Schwartz, a statistician attached to the Department of Surgery. The work has proceeded along two lines: 1) experimental, and 2) the continuation of a gathering of clinical data.

The experimental work has been directed towards a more critical evaluation of electrodes and the accuracy of placing electrodes. This is being channeled to the development of an intragastric balloon containing five electrodes which will cover the representative areas of the stomach and which will be synchronously recorded. This is being done in conjunction with x-ray verifications of the position of the electrodes. Also, an attempt is being made to study quantitative electrical form.

The clinical investigation has been concerned with the studying of an unselected group of asymptomatic patients with the purpose of a long term follow-up in correlating the electrogastrogram with the development of objective gastric pathology. A second clinical phase has been the development of a more objective method of interpreting the electrogastrogram recording by the use of a punch card system of punching characteristics and with the aid of a statistician who is working out a statistical method for the interpretation.

Dr. Robert Grinnell and Dr. N. Lane have continued their study of adenomatous polyps and papillary adenomas of the colon and rectum. The results of analysis of patients with squamous cell carcinoma of the anus have been published and the distal spread of carcinoma of the rectum and rectosigmoid has been described.

Dr. Daniel M. Shapiro, together with Dr. L.S. Dietrich of the Department of Biochemistry, Dr. Maurice Shils of the Department of Surgery, and Dr. Peter Wolkonsky of the Department of Medicine, has continued studies toward the two principal objectives of their research program: 1) to explore the feasibility of utilizing quantitative biochemical differences between normal and neoplastic cells as an approach to cancer chemotherapy; and 2) to study the possibility of eradicat-

ing neoplastic diseases by the simultaneous or sequential administration of combinations of chemical compounds selected upon this basis.

Further attempts at documenting quantitative biochemical differences between the 755 mammary adenocarcinoma and the normal tissues of the C57 mouse revealed the tumor to possess a relatively low coenzyme A concentration. On this basis, three pantothenic acid or pantetheine analogues (omega-methyl pantothenic acid and omega-methyl pantethine and bis-(beta-pantoylaminoethyl)-disulphide) were evaluated for carcinostatic activity, the best of them being bis-(beta-pantoyl-aminoethyl)-disulphide.

In anticipation of their eventual sequential blockade of the Krebs citric acid cycle in tumor tissue, the compound fluorocitrate was evaluated and found to possess carcinostatic activity. In vitro data reveal the mechanism of action of fluorocitrate to be that of blocking the utilization of citric acid. Additional in vitro data comparing fluorocitrate and fluoroacetate document interesting and probably important differences placing emphasis on fluorocitrate as a valuable compound in attempts at the multi-combination chemotherapy of cancer.

Sequential blockade attempts with a folic acid antagonist and a methionine antagonist revealed synergistic activity of this combination against the growth of the 755 mammary adenocarcinoma. The latter two compounds have also been evaluated in combination with a previously proved quadruple carcinostatic combination (8-azaguanine + testosterone + deoxypyridoxine + a riboflavin antagonist), with the demonstration of the advantages of employing large numbers of anti-metabolites in combination. The six compound combination results stress the distinct possibility of obtaining increased anti-tumor effect with decreased toxicity due to emphasis upon the "multi" aspect of combination therapy -- it would appear that, the greater the number of anti-metabolites, selected wherever possible on the basis of known or anticipated quantitative differentials, the greater the therapeutic index attained.

Dr. C.D. Haagensen, together with Drs. Michael Heidelberger, Daniel Moore and Otto Plescia have continued experiments to isolate, identify and characterize the agent which produces mammary cancer in mice. Experiments have shown that the agent is present in greatest amount in mouse milk; indeed, preparations of milk diluted down to 10^{-11} are active when they are injected into peritoneum. A whole series of such dilution experiments have shown the best manner in which to secure the agent and prepare it for inoculation. During the year it has been discovered that the agent is not visible, as had hitherto been thought, in the electron microscope, because treatment of milk preparation with a chelating agent clears the electron microscope preparations of all visible bodies, yet leaves the ability of the preparation to produce mammary cancer undiminished. Experiments are in progress to separate the agent from other proteins by means of starch column electrophoresis. To provide material for the research, an animal colony of pure bred strains of mice totaling about 9000 mice is maintained. This colony has also been useful in helping to establish strains for other research workers.

During the current academic year Dr. Raffaele Lattes and his associates in the Laboratory of Surgical Pathology have worked on the following projects in the area of cancer research: Dr. Carl Feind has completed a systematic study of the surgical specimens from 130 cases of elective radical neck dissections done for malignant diseases of the head and neck. He found that in 47 per cent of these cleared surgical specimens there were lymph nodes which contained metastatic tumor. This significant observation points to the necessity of neck dissections even in the absence of clinically demonstrable lymph node metastases.

Dr. John Pickren has continued his study of serial sections of axillary lymph node dissections from patients with carcinoma of the breast, which were reported negative on routine histological sections. In 18 per cent of the cases studied so far (35 cases) microscopic deposits of cancer cells were discovered. This study confirms findings of others and emphasizes the difficulty of evaluating the extension of malignant disease by the usual routine histological sections.

Dr. Nathan Lane has completed a study of 11 cases of squamous cell carcinomas of the upper respiratory tract and digestive tract. These tumors are associated with bulky pseudosarcomatous growths and have generally been mistakenly classified as some type of sarcoma.

Dr. R. Lattes, in collaboration with Dr. J. Malm, has analyzed 106 adults with malignant melanomas of the skin who have been followed for five years or longer. Some important features brought out by this study include: a) 26 per cent of the patients were alive without recurrence after five years; b) when a regional lymph node dissection was done and no metastases were found, 57 per cent of the cases survived five years or longer. Only 15 per cent of the patients with regional lymph node metastases survived for five years or longer; c) certain types, such as the subungual melanoma and the "freckle" type of melanoma have excellent prognosis.

Dr. R. Lattes, in collaboration with Mr. Saran Jonas, has reviewed the histological type and the follow-up of 26 patients with thymoma. An attempt was made to classify these tumors from a histological point of view and to correlate the pathological findings with the clinical prognosis. This study confirmed the view proposed by Castleman that thymomas are often locally invasive but metastasize only exceptionally.

The Histochemical Research Laboratory, under the direction of Dr. Gabriel Godman, has carried forward an active investigative program during the past year. Drs. C.A.L. Bassett, David P. Bloch, Arlene Deitch, and Henry Grossfeld, Miss Priscilla Goettler, and Mr. Charles C. Morris participated in various aspects of this program. It had previously been shown in the laboratory that DNA and histone are simultaneously doubled in amount during the interphase period preceding cell division, and that the histone is present in relatively constant amount with respect to DNA per cell. Further cytophotometric studies with a method devised in this laboratory in which the methyl green binding and Feulgen techniques for estimating DNA content, and the fast-green method for histone, are applied successively to the same cell have disclosed important differences in the desoxyribonucleoprotein complex of rapidly proliferating cells (called autosynthetic) as compared with non-dividing, presumably functional cells (called heterosynthetic). De-

phase cells, but did not occur in diploid autosynthetic or dividing cells. This was found to be due to the presence of a competing nonhistone protein, presumably the s-called "residual protein". Elimination of the inhibiting protein groups restored methyl green uptake.

The parallel increases in Feulgen and methyl green staining DNA during the period of doubling before mitosis of autosynthetic cells also indicate that no stage during DNA synthesis is characterized by an abundance of relatively unpolymerized DNA intermediates, which would be revealed by their decreased methyl green binding. In another interesting study, measurements of individual infected cells of the human viral skin papilloma at various stages in the intracellular lesions, have shown that as intranuclear infection progresses, there is a marked augmentation in the production of DNA to polyploid levels which is apparently ultimately shifted to the virus-containing inclusion. Studies are in progress to determine whether the increase occurs in regular multiples, and also to establish the relationship of the additional DNA to the formation of histone protein.

The cytological characteristics and localization of nucleic acid, glycogen, phosphatase, and changes in dye-binding in regenerating sarcoblasts at successive stages of their differentiation are being studied. In collaboration with Dr. Erich Hirschberg of the Department of Biochemistry, a sharp increase of pentose-nucleic acid (RNA) was found to occur in experimental rabbit muscle infarcts 4 days after injury corresponding to the appearance of undifferentiated sarcoblasts whose cytoplasm is rich in RNA. This increase appears to be sustained during the period of differentiation, when specific cytoplasmic protein is being formed.

Preliminary to cytochemical work on the cultured neuron, an adaptation of the method of freezing-dehydrating suitable for tissue cultures, required elaboration. The technique developed involves quenching the cells in isopentane at the temperature of liquid nitrogen, and "substitution" in ethanol at -40° C. This preserved cell morphology excellently, and left most constituents, including proteins, in optimal condition for cytochemical tests.

alkaline phosphatase and other characteristics of osteoblasts. With continued residence in vitro over long periods, especially in transplanted cultures, the population of phosphatase-positive cells is lost. Experiments are being conducted to induce the reappearance of cytoplasmic alkaline phosphatase and osteogenicity in these and other mesenchymal cells by various means. The acid mucopolysaccharide production of different forms of bone and of cartilage in mass cultures is also under study.

In collaboration with Dr. Levy of the School of Dental and Oral Surgery, it was found that exposure to crystalline Lathyrus toxin causes the formation of chordomas in Amblystoma larvae. A description of these experiments, and their interpretation in the light of the known action of the toxin was published.

Successive methyl green binding with and without acetylation of protein basic groups and Feulgen and fast green staining were used to determine the presence and state of DNA and histone in the specific bodies of lupus derivative from cell nuclei. The previously reported depolymerized state of DNA in these bodies and nuclei was confirmed. However, it was found that histone could not be detected in the LE cells and bodies, at any stages of their formation. This appears to be a unique instance of the disappearance of stainable histone from nuclear material with preservation of altered DNA. This evidence points to the possibility that the histone protein moiety of cell nuclei is attached and degraded by the circulating lupus factor, and is worth further study.

The Laboratory for Cell Physiology, under the direction of Dr. Margaret Murray, is completing its most active and most populous year to date, with so many investigators (8) that space and equipment are once more at a premium notwithstanding its recent expansion (4 years ago) into new quarters.

Research interests, which are concerned at long range with problems at the cellular level approached by the methods of tissue culture, may be grouped for the current year under three headings: 1) Factors governing the health of the normal cell, its differentiation and function. 2) Extension of existing culture tech-

niques for application to emerging requirements. 3) Various oncological problems.

In the first category an important contribution was made by Mrs. Helena Benitez through the collaboration of Dr. D.W. Woolley of the Rockefeller Institute for Medical Research. This suggests a possible mechanism by which the vasoconstrictor serotonin and certain of its antagonists may act to bring about mental disturbance. In 1951 it was shown by Lumsden and Pomerat that normal oligodendrogliocytes have a pulsatile function, the contractions being temporally of the order of those in smooth muscle. Our tests on human fetal oligodendroglial cells in vitro show that these respond to serotonin by entering into prolonged contraction, as do various types of smooth muscle cells in vivo. Two serotonin analogs, medmain and 1-methylmedmain, which antagonize its action on smooth muscle, also antagonize its effects on oligodendroglia. Lysergic acid diethylamide, however, which releases serotonin-induced contraction in smooth muscle of artery and uterus, seems to intensify the contraction produced in oligodendrogliocytes. When applied in the absence of serotonin, LSD has a relaxing effect which may obliterate normal pulsation.

Dr. John Paul (Rockefeller Travelling Fellow from the University of Glasgow) has shown that cobalt exerts a differential toxicity upon cultured dorsal root ganglion cells as compared with their supporting cells and with explanted skin, heart, and liver tissues. The characteristic appearance of the poisoned cells suggests a profound disturbance of the neuron's osmotic relationships.

Dr. Arlene Deitch has undertaken a cytological and cytochemical study of nerve cell differentiation in vitro, in cooperation with members of the Histochemistry Laboratory. She is presently concerned with the changing pattern of cytoplasmic constituents, especially the distribution of Nissl substance in the living cell, which she is recording by phase contrast photomicrography.

Mrs. Edith Peterson, who is investigating the nutrition of the neuron by the use of chemically defined media, has found that a synthetic medium of Parker and Healy which is sufficient to maintain fibroblasts and allow them to prolif-

erate will not support dorsal root ganglion cells without the addition of tissue extracts. Her recent work on myelin formation in vitro and the circumstances surrounding it has been presented by invitation at three international medical congresses. Her advice and help have been invaluable assets to our whole group who are studying nervous tissues in vitro.

In the subject of the second category, several advances have been made. Dr. Deitch has successfully adapted a freezing-substitution method of fixation to tissue culture preparations. Mrs. Peterson and Dr. Deitch, with other members of the laboratory, have shown that the type of glass is an important factor in the maintenance and survival of long-term cover-slip cultures of highly differentiated tissues such as nerve and muscle. Mrs. Ramirez, with the assistance of Dr. Bassett, is developing a strain of normal adult rat fibroblasts for use in modified mass culture experiments. Dr. Stanley Crain is accommodating existing cover-slip culture methods by chemical and mechanical means to further development of his work on electrical activity of single, intact neurons in vitro. Dr. Lasfargues, who has recently spent some time at the Strangeways Laboratory in Cambridge, England, learning methods of organ culture in vitro, is adapting these to his problem of modifying normal mouse mammary tissue by direct application of the Bittner agent. Mrs. Viveca Björklund is learning standard methods of tissue culture for adaptation to use in immunological studies on human tumors in vitro, wherein she will assist Dr. Bertil Björklund on their return to the Karolinska Institute, Stockholm.

In the third category (that of cancer research) efforts have continued, in collaboration with Drs. Arthur P. Stout and R. Lattes, to extend existing diagnostic criteria for human neoplasms. Also continued is the program previously undertaken with the cooperation of the Department of Neurosurgery and of Drs. Alfred Gellhorn and Erich Hirschberg, to attempt a systematic exploration of chemotherapy for the human glioblastoma multiforme. Tissue cultures are made from biopsy material taken at craniotomy and are subsequently exposed to poten-

tial carcinolytic agents; this is followed in suitable cases by clinical trial of agents selected on the basis of their destructive action on the patient's tumor in vitro. So far the most interesting group of compounds used in this investigation has been a number of acridine derivatives, which destroy glioblastoma tissue in vitro at exceedingly high dilutions. Comparative studies in vitro and in vivo on a transmissible mouse glioma also are being pursued. In general, correlation between the effects of specific chemical agents on the mouse tumor and on the human tumor is poor.

With Dr. Bertil Björklund, Visiting Fellow in Medicine, Mrs. Björklund is conducting preliminary studies on the effects of an immune factor upon human normal and neoplastic cells in vitro. This destructive agent is obtained from the serum of a horse which has been immunized against a variety of human cancers.

Dr. Etienne Lasfargues, formerly of the Pasteur Institute at Garches, has undertaken in collaboration with Dr. Dan Moore a project designed to determine whether the Bittner milk agent can be sustained or proliferated in cultured tissue and whether malignancy can be induced in normal tissue by the introduction of the agent. He has succeeded in the first step, which involves obtaining characteristic early mammary differentiation in cultures of fetal mouse skin.

Surgical Service, Francis Delafield Hospital

During the past year a continuation of the studies relating to the nutritional importance of the small intestine has been effected in the animal laboratories of Columbia University and in the Delafield Hospital. Previous work by Dr. Arnold J. Kremen and his associates has demonstrated that the distal half of the small intestine appeared to be a most important segment for absorption of fat and protein materials. Further confirmation of this thesis has been obtained during the past year in the animal laboratory. These studies have been pursued by Dr. Isio F. Wasserman with the assistance of Miss Annie M. Treacy.

A continuation of the studies by Dr. Cushman D. Haagensen and his associates on the routes of spread of mammary carcinoma has been carried out. Studies con-

tinue to demonstrate a strikingly high percentage of metastases to the internal mammary chain from cancer of the breast.

Within the Department of Surgery at the Delafield Hospital, Dr. Otto Lilien has been able to demonstrate tissue specific antigens and to produce tissue specific antibodies particularly to thyroid tissue. This has been a very important demonstration wherein Dr. Lilien has been able to show that thyroid tissue can be destroyed specifically by the injection of such antibodies. Similar work has been carried on in an effort to demonstrate antibodies to pancreatic tissue, various sorts of tumors, and also to a pituitary tissue. In some of this work, Dr. Lilien has been assisted by Dr. Melvin Horwitz.

In the clinical field further efforts to improve therapy of cancer of the esophagus have been carried out. A program has been instituted wherein patients with carcinoma of the thoracic esophagus have been treated initially with a high dose of radiation administered by the supervoltage techniques. At the completion of this therapy they have been operated upon and the esophagus removed. It is too early to appraise results of this approach to the problem except to state that it is technically possible to remove the esophagus after it has been heavily irradiated.

On the Head and Neck Service at the Delafield Hospital, Dr. Varham Bakamjian has been continuing the work in development of plastic techniques for reconstruction of surgical defects following extensive excision for cancer in this area.

During the past year, a start in the field of chemotherapy for solid tumors has been made. A preliminary screening study attempting to treat solid visceral cancer with thioguanine has been underway under the direction of Dr. Hugh Auchincloss and Dr. Fred M. Herter. To date the results of these efforts, although of interest, have failed to demonstrate any effective influence of this agent on solid tumors in the human.

A study attempting to define the effectiveness of per oral feeding preparations administered by gastrostomy and jejunostomy have been carried out by Dr. Clara Stein. These studies are still underway at present and results are not available.

Urology

Dr. Perry B. Hudson and his co-workers, Drs. T.D. Price, M. London, K.K. Tsuboi and M.E. Lombardo, have continued laboratory and clinical research in genito-urinary cancer.

During the past three years the studies have fallen into three main categories: (1) nucleic acid metabolism; (2) enzymology; and (3) steroid hormonal chemistry. Within these categories three kinds of experimental projects have been undertaken - those which are purely laboratory in nature, those which are clinical or clinical-pathological, and those which are combined laboratory and clinical problems.

In the laboratories devoted to nucleic acid chemistry a fluoroscope and Geiger counter for measuring ultraviolet absorption of chromatograms has been developed. Systems have been developed for enhancing the light absorption by various compounds in the chromatograms customarily used to separate substances important in the nucleic acid complexes. In addition, specific-activity determination has been greatly improved as a result of the development of an ultraviolet-photon counter. These improvements in technique, coupled with studies on the molar absorption relationships in chromatographic densitometry, have been incorporated into the study of nucleic acid metabolism in excised, whole human organs. Surgically removed human testis and kidney have been subjected to vascular perfusion techniques and subsequent analysis of tissue. These studies have by no means been completed but continue into the coming academic year.

Refinement of the techniques for the removal of non-steroidal pigments in urinary extracts and further development of methods for the extraction of steroids in blood have made possible the detailed analysis of human adrenal vein blood taken from patients with different kinds of malignant tumors. Also the analysis of steroids extracted directly from adrenal glands removed surgically has been accomplished. In order to clarify some of the data accumulated in this and other laboratories upon urinary steroid content, an in vitro perfusion of the dog kid-

ney has been undertaken. The principal purpose of these experiments is to determine the metabolism and conjugation of steroids in the change from blood to urine phase during the passage of these substances through a functioning kidney. Early results from these experiments indicate that the kidney is not capable of forming conjugates such as glucuronides, and that steroids added to the perfusate are not recovered as glucuronides from urine excreted during vascular perfusion of the isolated kidney.

The usefulness of glycyrrhizin in pharmacological maintenance of the adrenalectomized patient in steroid metabolic studies has been verified. This technique is still being employed in the department for certain feeding experiments which are readily performed during the post-adrenalectomy period.

The detailed studies upon the theoretical nature of inactivation and stabilization of purified human red cell phosphomonoesterase have been tentatively concluded. In connection with this, the fluoride inhibition and specific kinetic properties have been studied in connection with the purification of human red cell phosphomonoesterase. The differences between this enzyme and the prostatic acid phosphatase have been made clearly apparent, especially in experiments upon fluoride inhibition and heat denaturation. Although the general characteristics of enzyme foam fractionation have been the subject of extended study during the last 12 months, more emphasis has been placed upon denaturation studies on prostatic acid phosphatase, ion exchange chromatography, and the enzyme in the serum of patients with known metastatic cancer of the prostate. Although the demonstration of elevated amounts of prostatic acid phosphatase in the sera of patients with known metastatic cancer but normal phosphatase values by the usual clinical tests has been accomplished, the pharmacological and refrigeration techniques involved have, until the present time, not proved to be a practical clinical test. Improvements in the techniques used for elevating the acid phosphatase value of serum may soon result in a useful confirmatory cancer test.

The program designed to evaluate methods for detecting early prostatic cancer has been continued, and has resulted in advocacy that the transurethral and needle biopsy of the prostate gland be abandoned. The clinical survey of patients who are not suspected of having prostatic cancer is now more than one-half completed. In addition to the arbitrary biopsy of the posterior prostatic lobe in these unselected patients, a program for re-biopsy at two to three years intervals has been instituted.

In collaboration with Dr. Charles Crandall of the Department of Medicine, a clinical-laboratory study has been begun in an attempt to select patients with metastatic carcinoma of the breast who may react favorably to ovariectomy and adrenalectomy.

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SCIENTIFIC REPORT OF THE INSTITUTE OF CANCER RESEARCH

1955-1956

COLLEGE OF PHYSICIANS AND SURGEONS

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Scientific Report of the Institute of Cancer Research

1955 - 1956

College of Physicians and Surgeons

Columbia University, New York

Presented by the Cancer Research Coordinating Committee:

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Introduction

This is the fifth annual Scientific Report of the Institute of Cancer Research and, as in the past, it is dedicated to the individual donors, foundations and granting agencies of the Federal Government which have helped to support the studies reported. The facilities for communication of scientific data are so extensive, so easily available and so rapid that there would appear to be little need for a report such as this to present the work of members of the staff at Columbia University to colleagues throughout the world. In fact, it is recognized that the summaries which follow are too brief to offer complete objective evidence. It is felt, however, that a comprehensive picture of the work in progress here may be of interest to fellow scientists just as reports from their Institutions help us to form a clearer picture of the ramifications of research in the broad field of abnormal growth. The 164 bibliographic references of studies published in the past academic year provide detailed information for those who may wish to see more precise discussions of particular phases of the research described.

It is often difficult to discern changes in research emphasis over short periods of time, particularly if the reporter is working in the field himself. A five year interval, on the other hand, allows greater perspective to note trends. Some of the major movements which appear to this observer are:

1. The value of epidemiological research has received recognition particularly because of the world-wide interest in the demonstration of an increase of bronchogenic carcinoma among males. The impetus given to research on the etiology of this tumor through statistical collation of smoking habits in the male population and the relationship between cigarette smoking and lung cancer has been great. These investigations are only representative of others concerned with cancers of different anatomical sites. Geographical pathology, epidemiological study, occupational and personal habit correlations with specific tumors, all have pertinence to the possibility of cancer prevention. This is probably the most hopeful and productive approach to the problem of human neoplastic disease.

2. In the past five years, the potentialities and areas of usefulness of radical radical cancer surgery have been explored. The indications and limitations of this approach to therapy have now been reasonably well defined.

3. Supervoltage radiotherapy has also been introduced on a large scale in this five year interval and its value assessed. Although precise information on long term results is not available, it would appear certain that the various types of high energy sources provide sufficient quantitative advantages over conventional 250 Kv units so that they will continue as an integral part of radiotherapy.

4. Chemotherapy of cancer has expanded progressively at the laboratory and clinical level. The ever increasing emphasis on this therapeutic approach is less due to spectacular results achieved than a conviction that effective systemically administered treatment must be accomplished if the struggle against cancer is to be won. In the past five years progress has been made so that the use of drugs in the treatment of disseminated disease has greater applicability.

5. The trends in fundamental research are less easily described. This is so by the nature of the conventional definitions given to "fundamental" and "applied". Studies of basic cellular mechanisms which increase understanding of the behavior of animal or human tumors are obviously of fundamental importance but are often classified as applied. Recognizing the semantic difficulties involved it would appear that the most dramatic advances in basic research have been made in precise knowledge of nucleic acid biochemistry and its relevance to the tumor cell. More recently, nucleic acids and viruses are being linked not only structurally but with regard to their importance in the pathogenesis of abnormal growth.

6. One trend, clearly apparent to everyone in medical research, is that financial support now outstrips qualified research personnel. A discussion of the solution to this important problem is beyond the scope of these comments.

The points mentioned above constitute only a very partial view of the changes in the past five years. Within this framework, however, the work of the Institute of Cancer Research here has contributed knowledge and experience at the laboratory

and clinical level. Complacency and smugness are scarcely in order in a discussion of progress in cancer research; deprecation and hairshirts, on the other hand, are equally out of place. A reasonable view would appear to be that steady advances are being made which may ultimately diminish the threat which cancer poses for mankind.

Alfred Gellhorn, M.D.

June, 1956

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Biochemistry

Drs. Zacharias Dische and Renate Dische have continued their studies on serum and tissue mucopolysaccharides. It has been known for a long time that in certain diseases in which an accelerated protein turnover takes place there is an increase in the carbohydrate combined with the globulins of the blood plasma. Recent investigations have shown that this carbohydrate is a polysaccharide consisting of about equivalent amounts of galactose and mannose and of glucosamine. In addition, it contains a methylpentose, fucose, in amounts which vary from one protein fraction to another. There is considerable variation in the content of fucose from one individual to another and this sugar can be split off much more easily from the polysaccharide molecule than other constituents. According to recent findings, mucopolysaccharides of the same type are present in most animal tissues but significant differences in the relative amounts of the 4 individual sugars, particularly fucose, exist between different tissues. It seems possible, therefore, that during the development of malignancy the mucopolysaccharide fraction of the blood plasma may show characteristic changes in composition and/or structural properties which may be related either to the malignant process itself, or be due to a release into the blood stream of mucopolysaccharides from tissues which either are affected in their function during the development of malignancy or are subject to destructive processes due to tumor invasion.

It has been the general aim of this project to establish changes in the composition and structure of the mucopolysaccharides of individual protein fractions of the blood plasma of patients with various diseases, as well as experimentally induced metastatic tumors of rabbits. As a first step in this investigation a method was developed for the determination of the composition of the mucopolysaccharides in small quantities of plasma and for the determination of certain structural features of these mucopolysaccharides. This method consisted in a fractionated hydrolysis of the protein fractions by 10% trichloroacetic acid at 90° C. It was found that the rate of extraction was different for various constituents of the polysaccharide and

that fucose was extracted at a much higher rate than the other constituents. The ratio of the rate of the extraction of fucose and hexoses, on the one hand, and the 2 hexoses, galactose and mannose, on the other hand, was, therefore, adopted as a measure of certain structural properties of the mucopolysaccharide.

In the first series of experiments, it was determined to which extent various fractions of normal human sera differed either in the quantity or in the composition of the above mentioned structural factors from each other. The fractionation of the serum was carried out in these experiments either by the method 6 and 9, or the method 10 of Cohn. Each of the fractions obtained by these fractionation procedures differed not only in the content, but in composition and structure of the mucopolysaccharides. In normal rabbit serum, the content in fucose of the mucopolysaccharide was very much lower than in human sera. More recently, continuous flow electrophoresis has been adapted to the fractionation of human and rabbit serum proteins to obtain greater resolution of the fractions and to collect sufficient material for chemical analysis in a relatively brief time.

In a second series of experiments, a number of sera from patients with various forms of malignant and other diseases were analyzed, using the method 10 of fractionation. It has been found that every type of disease is characterized by a change in the distribution of the mucopolysaccharides between the fractions II, I, III, and fractions IV, V. These variations in distribution are accompanied by changes in the mucopolysaccharides which manifest themselves in the change in the relative rate of extraction of fucose, and, in some cases, in the relative rate of extraction of galactose and mannose. The results suggested that changes in the mucopolysaccharide in malignancy may be related to functional changes in certain specific organs like the liver and kidney. An examination of the mucopolysaccharides in 7 cases of liver cirrhosis and 5 cases of nephrosis showed constant changes. In liver cirrhosis, in the fraction IV, V there was a significant increase in the fucose content of the mucopolysaccharide to such an extent that the lowest value was far above the highest value obtained in normal sera. In 5 cases of nephrosis of children there

was a sharp decrease in the content of the mucopolysaccharides in fraction IV, V associated with a very low content in fucose. These findings seem to indicate a possible correlation between functional changes in these organs and certain structural properties of the mucopolysaccharide of fraction IV, V, and experiments are planned to induce liver and kidney injury in animals and to determine their effects on the mucopolysaccharides of the serum. As a preparatory step for the investigation of the effect of experimentally induced bone metastasis in rabbits, a study was begun of the neutral mucopolysaccharides of rabbit bones.

Among the several researches under way in the Biochemistry Division at the Francis Delafield Hospital, under the general direction of Dr. S. Graff, are:

a) A study of the characteristics of altitude acclimatization, in collaboration with Drs. A. Barach, H. Bickerman, and G. Beck of the Department of Medicine. It is known that animals, including humans, can be adapted by suitable means to live under low oxygen tension. This study seeks to determine the biochemical, physiological, and anatomical characteristics of such adaptations, the role of diet, age, and other factors, and the limits and observable criteria of acclimatization which in many ways resembles the conditioning of athletes. It appears that tissues or cells partake of acclimatization by re-adaptation of oxygen-sparing, predominantly glycolytic, enzyme systems.

b) Closely dependent on the investigation outlined above is that of the effects of hypoxia on tumor growth in acclimatized animals. Evidence has already been brought forward to demonstrate the deleterious and possibly therapeutic effects of hypoxia on tumors. Tumors appear to have requirements for oxidatively produced metabolites without which they cannot survive, and to the lack of which they cannot be acclimatized, whereas normal, adult, differentiated tissues are in large measure free of such rigorous requirements and can be adapted (acclimatized) to the satisfaction of their energy requirements by glycolytic pathways.

c) We have succeeded in developing a system for tissue culture whereby the replicating cells are always kept in constant logarithmic growth phase rather than in alternate feasting and fasting as obtained in static flask, or tube cultures. This system, which in some ways is the equivalent of a heart, lung, kidney preparation lends itself readily to metabolic turnover studies which are under way, and which have already yielded information on intermediary metabolism far less equivocal than that obtained from the whole animal in vivo. This system, furthermore, can produce controlled growths massive enough to make feasible the application of N^{15} to such studies.

d) We have succeeded in rounding out and completing the systematic synthesis of a number of substituted benzimidazoles, benzotriazoles, and quinoxalines which bear some structural correspondence to the naturally occurring purines and pyrimidines. Of these 75 new compounds, a number have been found to have interesting and possibly useful biological properties. Of particular moment is the sharp specificity of 4-methoxy-nitrobenzotriazole which is relatively inactive against the early dividing frog embryo, but which is acutely inhibitory in high dilution against late embryos in which differentiation and synthesis have commenced. The use of this compound demonstrates not only the distinction between cellular growth and cellular division without growth, but also the possibility of designing and synthesizing compounds with great specificity against particular enzyme systems.

A number of the compounds are strongly mutagenic at less than lethal concentrations in bacterial systems (Drs. F.J. Ryan and Sheldon Greer, Department of Zoology, Columbia University), and may serve as powerful tools in the elucidation of metabolic pathways in these organisms.

Some years ago, Tamm, Horsfall, and Folkers showed that a number of symmetrically di-substituted benzimidazoles are active as inhibitors of influenza virus. Our new compounds have been under study in the virus laboratory of Prof. P.K. Smith, George Washington University Medical College. He has reported to us that three of the new compounds we have supplied him are outstanding inhibitors of poliomyelitis

virus in tissue cultures and that he is now extending his experiments in multicellular systems. We shall continue to cooperate with him.

e) We have, along with many other laboratories, been interested for some time in the role of the nucleic acids in cellular events and the possible variations in structure of the nucleic acids reflecting biological or metabolic status. We are approaching this problem in several ways, analytically, preparatively, and synthetically. We are able to characterize both ribo- and deoxyribonucleic acids in tissues without prior isolation in terms of nucleotide content. We have isolated, and are now investigating, what appears to be compounds of nucleotides with amino acids. The synthesis of similar compounds is in progress.

f) McDowell showed some time ago that leukemia which is endemic in the C58 mouse can be markedly decreased in incidence by foster-nursing the C58 young against old, but not young, mature Storrs-Little mothers. We have in turn obligated ourselves to the search for, and the characterization of, the active anti-leukemia agent in the milk of old Storrs-Little mice. We are making progress in this area in spite of great difficulties; the agent is present in only small amounts, and rather large quantities of it in terms of milk are necessary for the demonstration of biological effects. Our most recent results indicate that the substance sought is of low molecular weight, i.e., it is ultrafilterable. The principal investigators are: Drs. Samuel Graff, Morris Engelman, Horace Gillespie, Victor Ross, Kathe Liedke, Ada Graff, Aaron Freedman, and Kenneth McCarty.

Dr. Stephen Zamenhof has continued, in collaboration with Dr. Hattie E. Alexander of the Department of Pediatrics, his studies on the resistance of the transforming principle (heredity determinants, DNA) to mutagenic, carcinogenic and carcinostatic agents. It was found that the individual heredity determinants differ in their average resistance to various mutagenic and carcinogenic agents, especially ultraviolet irradiation and nitrogen mustard. A resistance, characteristic for a certain heredity determinant, is retained on reproduction. Within each average resistance, there is a whole range of resistances of individual molecules, each of

which, on reproduction, again assumes a whole range of resistances.

In another project, Dr. Zamenhof has continued his studies on introduction of unnatural (halogenated) pyrimidines into DNA of E. coli. It was found that such introduction can also proceed without de novo synthesis of DNA , that the process is readily reversible and that in this respect the composition of DNA is merely a function of the environment. In another study, the inhibition of growth of certain strains of E. coli by natural nucleosides has been determined and a mechanism of this inhibition has been proposed.

Dentistry

Research in the Department of Dentistry of the Francis Delafield Hospital continues to be concerned primarily with the clinical and laboratory evaluation of various drugs and a number of oral pathology problems. A few of the studies mentioned in last year's report have been brought to completion and appear as literature references in this year's bibliography.

Drs. J.D. Piro, E.E. Sproul, E.V. Zegarelli, and A.H. Kutscher have continued their previously published studies evaluating a series of new local anestetic agents (Mizzy 285 and Pyribenzamine HCl) by intra-oral injection into the mucobuccal fold areas. The following have also been completed: studies in humans of the subcutaneous pain threshold with pressure using the Hardy-Wolff-Goodell algesimeter; a study of the effect of radiation exposure to the head during oral roentgenography on the cellular elements of the blood; killing-time studies on Bradosol in vitro against Monilia albicans which indicated marked activity of possible clinical import; a study of some of the problems encountered during mass dental extractions in adrenalectomized patients; a study of the histopathology of oral pigmentations and associated intestinal polyps in Jehger's syndrome; a pictural atlas of this latter disease is in preparation; histopathological studies dealing with familial white folded hypertrophy of the oral mucous membranes; a study to investigate the effect of a hydrocortisone ointment on chronic gingivitis.

A number of additional investigations are in progress or are being prepared for publication. These include: studies concerning the Hardy-Wolff-Goodell Radiant Heat Pain Threshold Apparatus and Technique; evaluation of the effect of analgesics on the pain threshold; clinical evaluation of phenaphen as an analgesic agent; continuation of a previously published disc sensitivity study to include the newer and also the previously available antibiotics in terms of all organisms isolated in the bacteriological laboratory; continued evaluation of a reported relationship between the occurrence of torus palatinus and breast or uterine carcinoma; evaluation of various denture adhesives; evaluation of sitosterol in the therapy of oral keratoses;

studies directed at demonstrating a non-inert activity of India ink; and studies to evaluate the effectiveness of pre-injection intra-oral topical anesthetic techniques.

Gynecology

Research in Cancer in the Department of Obstetrics and Gynecology has continued along the general lines laid down in previous years.

The work in the Department embraces two large fields, each with multiple aspects and a number of smaller, more defined but no less important projects. In the former class are first the studies of the steroids and their metabolism and in the latter the investigation of the biology and biochemistry of the degree of differentiation of tumors. Beside these composite projects, work is being done on the radiosensitivity of cervical cancer, on the transplantation of human tumors to animal hosts, and on the morphology of the ovary in the presence of gynecologic malignancy.

I. The steroid hormones and cancer

The work of Dr. Seymour Lieberman and his associates has been directed toward basic problems in the synthesis and interconversion of the steroids as well as in certain special biological aspects in their behavior. The general program may be outlined under the following subheadings:

1. Investigations into the nature and amounts of 17-ketosteroids excreted by (a) normal men and women to establish normal values and variability from day to day; (b) cancer patients treated with chorionic gonadotrophin and ACTH; (c) patients with Kimmelstiel-Wilson syndrome; (d) normal individuals treated with chorionic gonadotrophin or estrogen. The administration of chorionic gonadotrophin to 7 normal women and 5 normal men was accompanied by an increase of almost 100 per cent in the excretion of 11-oxygenated 17-ketosteroids in the urine. Three normal women have shown a similar response upon treatment with estrogen (Drs. J. Bitman, R. Vande Wiele, A. Granitsas, Messrs. M. Goldstein and J. Marquez).

2. Investigations into the nature of the corticoids excreted during pregnancy and by patients with Cushing's disease, adrenal virilism and Kimmelstiel-Wilson syndrome. The urinary metabolites excreted following the exogenous administration of 21-desoxyhydrocortisone and 21-desoxytetrahydrocortisone have been iso-

lated and identified. In addition, the urinary steroids excreted by a patient with a salt-losing syndrome have been examined (Dr. J.P. Rosselet and Miss Lillian Overland).

3. Investigation into the nature of urinary metabolites related to the newly discovered adrenal hormone, aldosterone. A sensitive technique capable of estimating 10 gamma of aldosterone or its urinary metabolites has been devised. With the help of this procedure it has been possible to isolate three new urinary metabolites of aldosterone (Dr. Stanley Ulick).

4. Investigations into the in vitro metabolism of C_{21} steroids by homogenates of mammalian liver (Dr. J.P. Rosselet and Miss Lillian Overland).

5. Investigations into the biosynthesis of steroid hormones by means of perfusion of isolated glands or homogenates of adrenal and placenta with C^{14}-labeled materials. The intermediacy of pregnenolone in the biosynthesis of progesterone from cholesterol has been demonstrated with adrenals and placenta. Using similar methods, it has not been possible to demonstrate the biosynthesis of compounds B and F by human term placenta. Seven possible intermediates between cholesterol and pregnenolone have been synthesized and are being tested for their possible role in the biosynthesis of the steroid hormones (Dr. S. Solomon, Mr. P. Levitan and Miss C. Cassanueva).

6. Investigations into the biosynthesis of the steroid hormones by means of homogenates of ovaries, follicles and corpora lutea with C^{14} materials. The intermediacy of pregnenolone in the biosynthesis of progesterone from cholesterol has been demonstrated in cow ovaries and cow corpora lutea. The biosynthesis of estrogens from progesterone by whole ovaries has not been demonstrable. Using bovine or human ovarian follicles it has been possible to prove the conversion of cholesterol to pregnenolone (Drs. R. Vande Wiele, S. Solomon).

7. Preparation of steroid-protein complexes and investigation of their chemical, immunological and biological properties. Three conjugates of testosterone and cortisone with bovine serum albumin have been prepared and their chemical prop-

erties have been studied. Rabbits, when immunized with each of these three conjugates, produced antibodies which are specific to the steroid haptenes. This is the first demonstration that the steroid hormones may serve as haptenes. Preliminary experiments have demonstrated that the antisera produced to testosterone bovine serum albumin have antihormone properties (Drs. B.F. Erlanger, Felix Borek and S. Beiser, Department of Microbiology, and Dr. F. Agate, Department of Anatomy).

8. Studies of methods for the synthesis and hydrolysis of steroid glucuronosides. Five already known steroid glucuronosides have been prepared in improved and practical yield. In addition, five new steroid glucuronosides have been synthesized and one of these has been shown to be identical with the main urinary metabolite of hydrocortisone, namely, tetrahydro E glucuronodate (Paul Levitan).

II. Differentiation in gynecologic neoplasms

The project designed to study the cytochemistry and biochemistry of differentiation in gynecologic neoplasms has continued along the lines described in the three previous years. A total of 208 tumors has been obtained for analysis since the initiation of the program on July 1, 1952. These tumors include 60 papillary serous and 29 papillary pseudomucinous ovarian cysts, 30 endometrial carcinomas, 47 cervical epidermoid carcinomas and 42 miscellaneous types. Specimens of each tumor have been distributed to participants in the program who are attempting to determine measurable or assessable cytochemical, biochemical and biological characteristics of malignancy that may be correlated with grade of malignancy.

Cytochemical studies have been continued, under the direction of Dr. Margaret E. Long, on ribonucleic acid, alkaline phosphatase, succinic dehydrogenase, glycogen and a number of other substances. During the year, two publications by Drs. Long and Howard Taylor have resulted from completed work in their phase of the project. One paper reports on the consistency of histological grade in ovarian serous papillary tumors, not only in different areas of the specimen but also in the structure of recurrences obtained months to years after primary operation. A second paper concludes that, in this same group of tumors, there is a statistically significant

increase in size and number of nucleoli, a measure probably of ribonucleic acid, with an increase in histological grade of malignancy of a tumor.

Current cytochemical studies have demonstrated sites of activity of the enzyme succinic dehydrogenase in the cells of all gynecological tumors and in endometrial glandular cells during phases of the menstrual cycle. It has been planned to relate such localized activity with grade of malignancy in the tumors and with the probable proliferative and metabolic associative functions of this enzyme system in cells.

The correlation of loss of differentiation in gynecological tumors with studies of their metabolic behavior has been continued by Dr. Helena de Roetth. The results indicate that the oxygen uptake in both air and oxygen increases with increasing malignancy. Evaluation of the percentage of epithelium in the tissue slices used for metabolic studies has shown, for ovarian papillary serous tumors, that the differences observed in oxygen consumption are real and not caused by variation of epithelial content of benign as compared to malignant tumors. Electrophoretic determinations of the soluble proteins of these tumors also can be correlated, in most cases, with malignancy. The incorporation of C^{14} glucose into the amino acids of the soluble and insoluble proteins is also under investigation.

A cytophotometric study of the nucleoproteins in individual nuclei has been set up by Dr. Saul Bader, for use in the study of the desoxyribonucleic acid distribution in the gynecological tumors. Quantitative data in terms of relative light absorption of DNA-Feulgen complex at 560 mμ in individual interphase nuclei have been collected on the basis of the measurement of approximately 3300 nuclei. At least 100 nuclei have been measured on each tumor specimen.

The histograms of the gynecological tumors indicate DNA distribution similar to those shown for control tissues. Variations in DNA distribution can be accounted for by the known mitotic irregularities which occur in tumors. The endometrial carcinomas of the different grades are essentially alike in their DNA spreads. The distributions of the ovarian papillary serous tumors suggest a parallelism with in-

creasing malignancy. Although increases in DNA distribution are not indicated by the data to be a specific criterion of malignancy, its possible association with invasiveness is to be considered.

Work on the nucleic acids and on certain enzymes of gynecological tumors has been continued by Dr. D.V.N. Reddy. Technical problems have made difficult the fractionation of human neoplastic tissues into their subcellular particulates. However, it appears that the RNA values per cell calculated on the basis of constancy of DNA per nucleus are consistently higher for the benign than for the malignant tumors of the ovary. The in vitro turnover of RNA ribonucleotides has been found to be considerably lower in the benign than in the malignant tumors.

D-amino acid oxidase activity of 33 ovarian and an equal number of other tumors has likewise been determined. Approximately 50 per cent of the benign ovarian tumors and all but 7 of the malignant tumors showed no oxidase activity. Closer examination of the problem showed that all of the 13 tumors tested, benign and malignant alike, inhibited D-amino acid oxidase. The inhibitor appears to be a characteristic of neoplasms since it is not present in normal animal tissues, such as rat liver or kidney. The inhibitor appears to be an enzyme, possibly a pyrophosphatase, which acts on the co-factor flavin adenine dinucleotide (FAD) of D-amino oxidase to yield the inactive flavin mononucleotide (FMN) and adenosine mono- or triphosphate. Although this mechanism was elucidated using extracts of adenocarcinoma 755, it is surmised that a similar or identical principle is operative in human tumors.

In the large study of the biology of differentiation in gynecological tumors, collaboration has continued with Dr. Michael J. Kopac and his group in the Department of Biology at New York University. Specimens of the tumor tissues being investigated here are sent to Dr. Kopac's laboratory, where various examinations are being applied, following such disciplines as: (1) the preparation and evaluation of isolated cell populations; (2) phase contrast study and photography under oil immersion optics; (3) high speed centrifugation and (4) in vivo cytochemistry with

III. Studies on the radiosensitivity of cases of carcinoma of the cervix

Dr. Saul Gusberg, with Dr. Margaret E. Long, Miss Jane Hill and Dr. Grace Herman, have continued their studies of radiosensitivity of cancer of the cervix in an effort to standardize testing methods for defining the most efficient treatment for cervical cancer in individual patients.

Cytochemical studies of nuclear changes under radiation have provided techniques for defining regressive changes in tumor vitality within several weeks of onset of treatment. These changes include: a) enlargement of the tumor cell nucleus; b) relative diminution in DNA as indicated by dilution of the chromatin masses; and c) relative increase in RNA as shown by increase in nucleolar size and number. These changes occur within several weeks of the onset of treatment before a clinical examination can reveal any significant change in gross characteristics. A small therapeutic test dose of radiation is given to measure this response in a standard way so that the spectrum of sensitivity response can be evoked and so provide a scale of reactions.

The clinical accuracy of this type of testing has proved to be high and this type of data can provide a sufficiently quantitative means of testing response to indicate either pure radiation treatment, pure surgical treatment, or a combination of both treatments for the primary tumor and its primary nodal depots. A recent analysis of our radiation failures in favorable stages of the disease has been used to outline the role that such radiosensitivity testing could play in the treatment of cervical cancer.

In addition, a study has been begun of the alleged host response as measured in normal vaginal cells according to the method described by the Grahams at the Massachusetts General Hospital. With the addition of this procedure it may be possible to explore the cytologic response of both tumor and host.

IV. Study of the morphology of the ovary in patients with gynecological neoplasms

In the past year, the ovarian histology laboratory has continued the collection of ovaries and the quantification by morphological techniques of the functioning tissue contained in these ovaries. At the present time 130 ovaries have been collected. Of these, fifty-three ovaries have been completely studied: cancer of the cervix, 19; cancer of the corpus, 1; fibromyomata uteri, 17; "chronic pelvic congestion", 4; cancer of the breast, 2; "cystic ovary", 2; normal, 8. Eighty-seven additional ovaries are in preparation.

This study should result in an increased knowledge of the human ovary, its functions and morphological characteristics as well as clarifying the influences exerted on the ovary by age and pathological conditions. A preliminary inference which may be drawn is that all the pathological conditions studied thus far seem to show a higher per cent of functioning tissue than the normal. This may prove of significance when considered in relationship to the known effect of the ovarian hormones on reproductive tract tumors of certain laboratory animals.

V. Studies on the transplantation of human gynecological neoplasms

The study of heterologous transplantation of human gynecologic cancers begun by Dr. Harold M.M. Tovell a year ago has continued. A total of 57 human gynecologic malignant tumors have been transplanted into conditioned laboratory animals. Conditioning of the host was accomplished by regular subcutaneous injections of small doses of cortisone and by total body radiation prior to implantation of tumor tissue.

Of the 57 malignant tumors studied there were 22 ovarian, 22 epidermoid carcinomas of the cervix and 13 endometrial carcinomas. In the entire series 24 per cent of the implants survived one transplant generation and 33% showed gross and microscopic evidence of growth for 2 or more generations, making a total number of successful transplants of 57%. No permanently transplantable tumors were obtained, although one tumor continued to show a vigorous growth for 7 generations prior to

A review of previous work done with the intraocular heterotransplants of malignant ovarian tumors was carried out with the purpose of correlating patient survival and results of heterotransplantation. On the basis of twelve tumors studied, with a patient follow-up of two years or more it was noted that the average patient survival time in those tumors that were heterotransplantable was one-half that of patients displaying non-heterotransplantable tumors.

VI. Clinical investigation on the therapy of gynecological cancer

The general plan set up four years ago, on the basis of agreement between the Radiotherapy and the Gynecology Departments, has continued to be the basis for the decisions on types of therapy. Considerable numbers of cases have now been treated in certain categories but the follow-up periods necessary for evaluating results have of course not yet elapsed.

Efforts continue to be made to compare the results of surgical and radiation techniques and of combinations of certain of these. Improvements in procedure, especially the availability of supervoltage x-ray and of rotational cobalt have introduced new variables which have made the methods of treatment more heterogeneous than had been hoped when the study was set up. It is planned that within the next few months a new series of interdepartmental conferences will re-define therapeutic policy for each type of gynecological cancer.

Medicine

The principal objective of this group has been to develop a broad program of research on medical aspects of neoplastic disease. This has encompassed three major activities which include: 1) the study of the natural history of certain cancers in man, 2) investigations on the pathological physiology of the human tumor bearing host, and 3) chemotherapy. These three categories are intimately interrelated for knowledge of the natural history of disease is a prerequisite for evaluation of therapeutic regimens and investigation of pathophysiology provides additional objective criteria which can be used in following the effects of chemotherapeutic agents. The table depicts the categories of activities as well as the professional personnel involved in their implementation.

Tumor Screening and Biology

A. Gellhorn, M.D.

E. Hirschberg, Ph.D.

E. Anglesio, M.D. **

Mechanism of Drug Action

J.D. Davidson, M.D.

P. Feigelson, Ph.D.

Clinical Chemotherapy

A. Gellhorn, M.D.

D. Choy, M.D.

C. Crandall, M.D.

A. Edwards, M.D.

G. Hyman, M.D.

J. Mijnssen, M.D.

H. Ranney, M.D.

T. Richter, M.D.

J. Ultmann, M.D.

Pharmacology

L. Reiner, M.D. *

A. Gellhorn, M.D.

E. Hirschberg, Ph.D.

Metabolism

P. Marks, M.D.

J. Bishop, M.D.

Hematology

G. Hyman, M.D.

J. Ultmann, M.D.

Myeloma

E. Osserman, M.D.

Biochemistry of Differentiation

E. Hirschberg, Ph.D.

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* Deceased November, 1955

** Visiting Fulbright Fellow from Italy

The chemotherapy program has included laboratory and clinical studies. The former consisted of investigations on screening methodology and screening against tumors in experimental animals, studies on the mechanism of action of carcinostatic drugs, and pharmacology. The clinical cancer chemotherapy research has been conducted primarily on the seventy-four bed medical ward and the out-patient department of the Francis Delafield Hospital.

Tumor screening and biology

a) Screening methodology - In last year's report, reference was made to an inter-institutional investigation of cancer chemotherapy screening methodology which involved a comparison of the performance of a wide variety of biological test systems. The results of this study, which were published as Supplement No. 3, 1955 of Cancer Research, indicated that experimental tumors constituted the most satisfactory and specific system for the selection of anti-cancer drugs of the types now available.

In reviewing this work, it appeared of interest to extend the study to include a biological system which had been omitted from the earlier investigation, namely normal mammalian bone marrow. Drs. Enrico Anglesio and Alfred Gellhorn therefore examined the effect of the twenty-seven compounds used in the earlier experiments on the bone marrow, intestine, liver, and kidneys of normal rats. Each compound was injected into at least five Wistar rats at maximum tolerated doses. Peripheral blood counts were determined daily and the animals were sacrificed six days after the completion of the series of injections.

The peripheral white cell count was significantly decreased by nitrogen mustard, urethan, 2,4-diamino-5-(3',4'-dichlorophenyl)-6-ethylpyrimidine, 1,5-diaminobiuret, and azaserine; a significant increase, conversely, was caused by the administration of 8-azaxanthine, 2,6-diaminopurine, ethionine, and β-2-thienylalanine. Qualitative changes in the bone marrow were caused by about half of the compounds, including both carcinostatic and non-carcinostatic agents. No effects of any compounds on the liver and kidneys were seen. The mitotic index of the small intesti-

nal epithelium was significantly changed by twenty of the twenty-seven compounds, but, interestingly enough, only the two antibiotics chloramphenicol and actinomycin caused a decrease, with rises of varying magnitude following the administration of the other agents.

In general, these results show again that there is no relationship between the anti-tumor activity of these selected agents and their effects in other biological systems and, consequently, that normal mammalian bone marrow does not lend itself well as a screening tool to the search for carcinostatic agents.

b) Experimental tumor testing - The central objective of the various laboratories across the country engaged in experimental cancer chemotherapy is the discovery of new agents of potential interest for the treatment of the human diseases. There are a number of different biological systems which lend themselves to this quest. There are, also, many varied reasons for the selection of particular compounds for evaluation.

In this laboratory, Drs. Alfred Gellhorn and Erich Hirschberg, together with Misses Shirley Drayton, Janet Hibbert, and Gloria Semente, have continued to test each new compound against a wide spectrum of transplanted mouse tumors. This spectrum comprises three mammary adenocarcinomas (755, Eo771, RC), a fibrosarcoma (S180), a transplantable brain tumor (glioma 26), a lymphosarcoma (6C3HED), a myelogenous leukemia (C1498), and the Ehrlich ascites tumor. In almost all instances, treatment of the tumor-bearing animals with the compound under test is started one day after transplantation and continued every day except Sunday. For the first five tumors in this spectrum, the evaluation of the carcinostatic effectiveness of the agent is based on a statistical comparison of the weights of the tumors removed from treated and untreated groups of ten to twenty mice each at the end of the experiment; for the last three tumors, the effect of the compound is measured in terms of prolongation of the survival time of the tumor-bearing mice.

The compounds to be tested are chosen, wherever possible, to represent structural features of interest in one of several unrelated classes of chemicals. At-

tention is currently focused on quinolines and acridines, on "one-armed" and "two-armed" nitrogen mustard derivatives, and on benzimidazoles and benzotriazoles. Each compound is tested at its maximum tolerated dose, as established by a five-day toxicity test on normal mice. If tumor inhibition is accompanied by excessive weight loss of the host animals, it is not considered to be significant. Almost without exception, the intraperitoneal route of administration is used.

The acridines, which include the well-known antimalarial quinacrine (atabrine), are essentially inactive when injected into tumor-bearing mice, although they exhibit striking carcinostatic activity in tissue culture (cf. below). The only tumor which responds to quinacrine to an appreciable extent is the Ehrlich ascites tumor, in which the compounds are injected directly into the fluid containing the tumor cells. Feeding experiments currently in progress suggest that the oral route of administration may be preferable for compounds of this sort. A series of quinolines, including the antimalarials chloroquine and camoquin, is under active investigation. Among the benzimidazoles, ring halogenation appears to be needed for carcinostatic activity, as shown by the inhibitory effect of 5,6-dichlorobenzimidazole against several tumors and the lack of activity of unsubstituted or differently substituted benzimidazoles. The "two-armed" nitrogen mustard derivatives as a group exhibit a fair amount of anti-tumor activity; a benzimidazole mustard, 2-(di-(2-chloroethyl)aminomethylene)benzimidazole, inhibits the growth of several dissimilar tumors in the spectrum and is therefore under current study from the point of view of toxicology and pharmacology in rabbits and dogs.

c) Experimental chemotherapy of brain tumors - The interdisciplinary investigation of approaches to chemotherapy of the malignant brain tumor, glioblastoma multiforme, has continued to engage the attention of Drs. Gellhorn and Hirschberg in collaboration with Dr. Margaret Murray and Mrs. Ruth Eising of the Laboratory of Cell Physiology and with Drs. Hartwell Thompson, Joseph Ransohoff, and J. Lawrence Pool of the Neurological Institute. This investigation comprises three general areas in addition to the in vivo screening of compounds against mouse glioma 26

mentioned above: tissue culture experiments with samples of this mouse brain tumor and of human brain tumors obtained at operation from a series of patients; clinical evaluation of promising agents; and determination of drug distribution in both mice and patients where analytical methods are available or can be developed.

Extensive testing has shown that acridines and quinolines, as a group, possess pronounced activity against the brain tumors in tissue culture. Certain benzimidazole derivatives exhibit carcinostatic activity which increases with increasing degree of substitution; for instance, 5,6-dichlorobenzimidazole and 5,6,7-trichlorobenzimidazole ribofuranoside are about twenty and two hundred times, respectively, as active as benzimidazole itself. The nitrogen mustard derivatives, in general, are relatively inactive under the conditions of these tests.

On the strength of their striking activity in vitro and of their widespread clinical application as antimalarials, quinacrine and camoquin (amodiaquin), representative of the acridines and the quinolines under investigation, have been selected for evaluation in a series of patients with far advanced glioblastoma. Quinacrine has been administered per os to about a dozen patients for varying periods of time without significant improvement. The number of patients treated with camoquin is too small to permit any evaluation at the present time.

The failure of quinacrine to inhibit the growth of experimental tumors in mice or to affect the clinical course of the disease in man in the face of its pronounced activity in tissue culture has led to a search for a reason for this discrepancy. The availability of a fluorometric assay method of sufficient sensitivity has made it possible to determine the quinacrine levels in blood plasma obtained from patients or experimental animals after various courses of drug administration. These experiments, conducted with Mrs. Martha Osnos, showed that the blood levels which could be built up by injection of maximum tolerated doses of the compound fell short of the minimum inhibitory concentration present in tissue culture feeding fluids. Analogous determinations of quinacrine levels in the cells of human or mouse tumors after treatment indicated that the concentration present in the cells

grown in vitro was five to ten times as high as in cells grown in vivo. The use of the oral route of administration, in preliminary experiments with mice, promises to lead to a greater accumulation of the drug in the tumor cells as well as to a greater effect of the drug on the growth of the tumor. A similar series of experiments with camoquin is in progress.

Mechanism of drug action

Dr. Jack D. Davidson has been concerned with investigation of the effects of anti-tumor compounds upon nucleic acid metabolism. These studies have been directed toward elucidating the mechanism of action of the compounds and the mechanisms of inherent and acquired resistance to the anti-tumor agents.

In the same program Dr. Philip Feigelson has investigated the effects of a number of purine analogs upon certain enzyme systems. He found that 8-azaguanine produces 50% inhibition of adenosine deaminase and xanthine oxidase at concentrations of 4×10^{-4} M and 5×10^{-5} M respectively.

This latter effect is capable of explaining many previously reported findings regarding 8-azaguanine and would be consistent with the anti-tumor activity of the compound being attributable to a guanine moiety deficiency. It is suggested that the deficiency results from inhibition of the xanthine oxidase catalyzed conversion of hypoxanthine to xanthine on a pathway of guanylic acid biosynthesis. Certain anti-tumor pyrazolopyrimidine analogs of normal purines have activity of a similar type.

A Tri-carb liquid scintillation counter has been available to this group for the past year and considerable effort has been expended on devising practicable techniques for counting C^{14}-labeled purines with this instrument. These efforts have been successful and a method of counting with 50 times the sensitivity of previous techniques has been developed.

Clinical chemotherapy

In the past two year period eight compounds have been evaluated in three hundred and thirty-eight patients with a variety of disseminated neoplastic diseases. A summary-at-a-glance is as follows:

Drug	Number of Patients Studied	Usefulness of Drug
6-Mercaptopurine, Purinethol(R)	87	Acute Leukemia, children
1,4-dimethanesulfonyloxybutane, Myleran	41	Chronic myeloid leukemia
Thiotriethylenephosphoramide, Thio-TEPA	79	Lymphomas, ovarian carcinoma
P-dichloroethylaminophenylbutyric-acid CB 1348, Chlorambucil	30	Chronic lymphoid leukemia
Prednisone, Meticorten	32	Acute leukemia, adults
Sarkomycin	20	None
Triethylenemelamine, TEM	34	Retinoblastoma
Acridines	15	Undetermined

The investigations of seven of the eight drugs have been summarized for publication. Only brief discussions will be presented here of those not summarized in last year's report.

a) Myleran. Twenty-one cases of chronic myeloid leukemia and 20 patients with other types of neoplasm were treated with Myleran during a 4 year period. Eighty-one percent of the group with chronic myeloid leukemia showed significant remissions over a period of up to 48 months. None of the patients with other neoplasms responded to Myleran. The objective of therapy is to bring the leukocyte count to normal range together with a decrease in visceral infiltration, decrease in thrombocytosis and a spontaneous rise in the hemoglobin. Myleran was found to be effective in patients in whom radiotherapy had become unsuccessful and the drug was of particular value in such cases. Whether or not it is superior to radiotherapy and should become the treatment of choice in chronic myeloid leukemia will require further experience.

The following table indicates the diseases studied:

Type of Neoplasm	No. of Cases	Hematologic Depression	Subjective and Objective Effect
Chronic myeloid leukemia	22	No	18 improved
Multiple myeloma	4	Yes	None
Reticulum cell lymphosarcoma	3	Yes	None
Bronchogenic carcinoma	2	Yes	None
Breast carcinoma	8	Yes	None
Carcinoma of rectum	1	Yes	None
Carcinoma of stomach	1	Yes	None
Melanosarcoma	1	Yes	None

b) Thio-TEPA (Triethylene thiophosphoramide). This drug has been evaluated in 80 patients with various neoplastic diseases. It was found to be of no benefit in carcinoma of the breast, carcinoma of the liver, carcinoma of the lung, malignant melanoma and miscellaneous carcinomas, nor was it of particular usefulness in lymphosarcoma. In patients with Hodgkin's disease, of which 10 were treated, 8 showed moderate or marked objective improvement and 5 showed considerable subjective improvement. Of 16 patients with reticulum cell lymphosarcoma 4 showed marked or moderate objective improvement and 3 showed subjective improvement. In carcinoma of the ovary, of 20 patients treated, 9 showed marked or moderate objective improvement while 11 reported subjective improvement. In the 80 instances in which the drug was used, 11 patients showed some form of bone marrow toxicity and 3 patients died during therapy as a complication of toxicity.

A summary table of the diseases studied and results follows:

Disease	No. of Patients	Moderate or Marked Improvement Objective	Subjective	Toxicity
Ca. of breast	7	0	0	2
Ca. of liver	2	0	0	1
Ca. of lung	3	0	0	0
Hodgkin's disease	10	8	5	2
Lymphosarcoma				
Giant follicle	2	0	0	0
Lymphocytic	6	2	1	1
Reticulum cell	16	4	3	3
Melanoma	6	0	0	0
Ca. of ovary	20	9	11	2
Miscellaneous	7	0	0	0

c) P-dichloroethylaminophenylbutyric acid, CB 1348, Chlorambucil. Thirty patients were treated with p-di-2-chloroethylaminophenylbutyric acid (CB 1348). Eighteen patients with symptomatic chronic lymphatic leukemia were given 20 courses of therapy. Twelve showed a significant fall in white blood cell count and in 5 of these, percentage of lymphocytes was decreased by 25% or more, together with a decrease in hepatosplenomegaly and lymph node enlargement. About 25% of the patients reported subjective improvement and return to normal activities. The results in 12 patients with various lymphomas were not encouraging.

The drug did not produce gastrointestinal side effects nor hepatic or renal complications. Severe leukopenia and thrombocytopenia occurred in one instance.

A tabular summary of the evaluation of this anti-tumor drug follows:

Diagnosis	Number	Objective Improvement
Chronic lymphatic leukemia	18	11
Giant follicular lymphosarcoma	1	0
Lymphocytic lymphosarcoma	5	2
Hodgkin's disease	6	1

d) Prednisone, Meticorten. The administration of the available chemotherapeutic agents to adults with acute leukemia has resulted in a low remission rate with little change in total survival time. Hill and Vincent reported a relatively high remission rate for patients with acute leukemia who received large amounts of fluorohydrocortisone. The use of prednisone and prednisolone which show less salt retaining activity than either cortisone, hydrocortisone or fluorohydrocortisone, would have obvious advantages from the standpoint of minimizing side effects of steroid therapy in very large doses. The present study was undertaken to ascertain the usefulness of massive steroid therapy in patients with acute leukemia and far advanced lymphomas. The patients may conveniently be placed into two categories: 1) those with subacute or acute leukemia, and 2) those with far advanced lymphomas.

Prednisone and prednisolone (in two of the patients early in the series cortisone was used) were given in doses of 0.250 gm. every six hours. Patients were placed on an ambulatory ulcer diet and antacids and about 3 grams of potassium chloride daily was given in the form of enteric coated tablets. In all but 3 pa-

tients the initial dose of steroid was of this order of magnitude, three patients have received trials of 75 mg. of prednisone before massive therapy was started.

A tabular summary of this clinical therapeutic experiment follows:

Diagnosis	Remission		No Effect	Major Toxicity
	Complete	Partial		
Leukemia				
Acute (16)	4	4	8	2
Subacute (3)	0	1	2	2
Blastic crisis of chronic myeloid leukemia (3)	0	1	2	0
Lymphosarcoma (8)	1	1	6	4
Other (2)	0	0	2	2

e) Sarkomycin. In the early Spring of 1955, there was much rumor and discussion about the therapeutic efficacy of the "antibiotic" Sarkomycin in disseminated epithelial tumors. It was very difficult to evaluate the Japanese data which, in the main, looked very poor. As a public service, rather than because of any conviction that the drug looked promising, a cooperative venture was undertaken with colleagues of the Sloan-Kettering Institute to evaluate the compound in human cancer.

The results of Sarkomycin therapy in a variety of widespread epithelial cancer fully justified our pessimism. Other than a high frequency of phlebothromboses at the site of intravenous injection and gastrointestinal toxicity, the material produced no evident effect. Our experience has been summarized and will appear in a joint publication with the Chemotherapy Service of the Memorial Center for Cancer and Allied Diseases.

f) Triethylene melamine, TEM. In January 1953, Drs. George Hyman and Algernon Reese started to treat all suitable patients with retinoblastoma by the combination of radiation and TEM. Combining x-ray treatment with a radiomimetic drug seemed to offer a good opportunity to reduce considerably the x-ray dose, which might then lead to a decreased incidence of late retinal hemorrhage and blindness, and an increased cure rate. Most patients treated were instances in which the eye

with the more advanced tumor was enucleated and the eye with the less advanced lesion was treated. The criterion always was whether or not useful vision could be retained if the tumor were arrested. If not, then, of course, enucleation was preferable.

In the past 21 months 34 eyes have been treated with radiation and TEM according to the method described here. Nineteen of these eyes have shown satisfactory regression. In addition, 8 eyes were treated in which the lesion showed a good response in the beginning but after a period of time, ranging from 6 to 8 months following the completion of the treatment, the tumors manifested active growth. Additional therapy to these 8 eyes was instituted, and in 3 instances the lesion has regressed satisfactorily.

In summary, it must be said that TEM therapy alone has not proved curative in the treatment of any neoplasm in the past, no matter how beneficial it may appear initially. No different end-result should be expected in patients with retinoblastoma. Consequently, the use of TEM alone has not been assayed in the present series. We feel, therefore, in view of the evidence obtained in the present study, that although TEM exerts a clear-cut, beneficial effect on retinoblastoma, its present role is in combination with properly administered radiation.

g) Quinacrine and Amodiaquin. In an earlier section of this report, reference has been made to the program on the chemotherapy of brain tumors. On the basis of experiments there described, quinacrine and amodiaquin have been selected for clinical evaluation in patients with glioblastoma multiforme refractory to further radiotherapy.

In the past 2 years, 15 patients have been studied, 11 receiving quinacrine and 4, amodiaquin. With the exception of 1 patient, none of those receiving quinacrine showed objective improvement. The observations with amodiaquin are, as yet, too preliminary to merit description.

As mentioned above, in the quinacrine treated patients pharmacological studies on drug distribution in extracellular fluids and various tissues were made. These

revealed that with maximally tolerated doses, chronically administered, the concentration of drug failed to equal the effective level determined in parallel in vitro experiments. For this reason quinacrine therapy of brain tumors has been abandoned. Similar distribution observations are being made with amodiaquin.

Metabolism

a) Alterations in carbohydrate metabolism associated with neoplasia - A large literature has accumulated relating alterations in carbohydrate metabolism to the neoplastic process. Disturbances in host-tissue protein metabolism associated with tumor growth have been described. However, alterations in glucose metabolism associated with neoplasia in human subjects have not been defined. Early studies have described an increase in fasting blood sugar as well as a decrease in glucose tolerance test in patients with various malignant diseases. These studies have been inconclusive, however, with regard to the proper isolation of the effects induced by neoplasia, primarily because secondary effects intervening in the course of the disease were neither controlled nor excluded.

In the present investigation Drs. Paul Marks and Jonathan Bishop have studied glucose metabolism in carefully selected patients with leukemia, lymphoma and clinically-localized carcinoma employing a technique of intravenous glucose tolerance test which permitted the calculation of the fractional rate of blood glucose disappearance. These patients were selected to ensure adequate dietary intake for at least two weeks prior to the test. The patients had no weight change during the 6 months preceding the test, no family history of diabetes mellitus, no other associated acute or chronic diseases and the test was performed in only those patients who were fully ambulatory.

Observations based on 86 glucose-tolerance tests in 33 patients with malignant disease and in 17 normal individuals revealed that the subjects with malignancy had a fractional rate of glucose disappearance which averaged 2.24 ± 0.71%/min. as compared with the normal individual whose mean was 4.19 ± 1.31%/min. This difference between the means for the fractional rate of blood glucose disappearance was significant.

This finding contrasts with the recognized high rate of glycolysis that characterizes metabolism of tumor tissue.

There was no correlation between the fractional rate of blood glucose disappearance (K_GD) and the height, weight or body surface area of the individuals. The fasting blood sugar of the patient group had a mean of 88 ± 12 mg.% and that of the control group 80 ± 9 mg.%. There was no significant difference between these means. The urinary glucose excretion during the glucose tolerance test did not differ significantly between the two groups. In addition to a smaller fractional rate of excess glucose disappearance, the patients with malignancy had a decrease in the net glucose disappearance from the blood during the course of the glucose tolerance test as compared with the normal group.

If the decrease in K_GD can properly be interpreted as a phenomenon related to the presence of a malignant process, one might expect, in the absence of extraneous factors, that this defect in carbohydrate metabolism will revert toward normal with extirpation of tumor or with effective chemotherapy. Serial observations have been made on 2 patients prior to, during and following effective chemotherapy. These patients were studied on the Metabolic Unit while on a constant diet and with complete nitrogen and electrolyte balance studies. In both patients it was found that the K_GD reverted to normal following therapy. This return to normal of the K_GD occurred while the patient exhibited negative nitrogen balance. One of these patients has now been followed through a second cycle of exacerbation, therapy and remission of disease with similar findings. In this patient, exacerbation of the disease was associated with a decrease of the K_GD to an abnormally low value. Following a second course of effective chemotherapy, the K_GD reverted to normal. In addition, 2 patients, one with cervical carcinoma and the other with carcinoma of the female breast, have been studied prior to and following surgical removal of the tumor. In these patients the K_GD following surgery has reverted towards normal.

b) Studies on biosynthetic pathways of ribose of ribonucleic acid in liver, muscle and tumor - (This project is being carried out by Dr. Paul Marks in cooperation with Dr. Philip Feigelson of the Department of Biochemistry.)

It has been shown that ribose phosphate is an intermediate in the pathway of glucose metabolism referred to as the pentose phosphate shunt. At least 2 possible series of reactions may be involved in ribose-5-phosphate synthesis by this pathway. These two series of reactions may be distinguished by the pattern of C^{14} labelling present in the ribose arising from specifically labeled precursors. Thus if ribose phosphate is synthesized primarily by decarboxylation of glucose-6-phosphate, the labeled carbon of ribose should correspond to the labelling in carbon positions 2 through 6 of glucose. If, on the other hand, ribose-5-phosphate synthesis primarily results by the series of reactions catalyzed by the enzymes, transketolase and transaldolase, the pattern of labelling in the resultant ribose would be quite different from that predicted for the decarboxylation reaction. Thus it has been shown that as a result of the reactions catalyzed by the enzymes, transketolase and transaldolase, C^{14} originally in C-1 of glucose would distribute itself to carbons-1 through 3 of ribose. These studies provide a basis for approaching the problem of the biosynthetic pathway of ribose and of ribonucleic acid. In the current study $C^{14}O_2$ as sodium carbonate was injected intraperitoneally into rats, and the glycogen and RNA of liver and muscle isolated. The glycogen was degraded to glucose and ribose was recovered from the RNA fractions. The glucose was degraded by the bacterial fermentation with Leuconostoc mesenteroides, ribose was degraded by bacterial fermentation with the Lactobacillus pentosus. Work to date has primarily established the validity of the techniques employed. In particular it has been shown that sufficient radioactivity may be incorporated into the ribose of RNA of both muscle and liver to allow for accurate degradation of quantities as small as 1 mgm. of ribose. In addition, it has been shown that the bacterial fermentation methods allow for the direct determination of the relative specific activity of each of the individual carbon atoms of glucose and of ribose. It is planned, by using variously labeled precursors including $C^{14}O_2$ and glucose labeled specifically in carbon positions 1, 2 and 6, to study the resultant pattern of distribution of C^{14} in the ribose of RNA of liver, muscle, regenerating liver and various tumors.

Hematology

In the past year Drs. George Hyman and John Ultmann have attempted to use the increased rate of red cell destruction, which Dr. Hyman has previously observed in patients with cancer, as an objective criterion in the evaluation of therapeutic measures. Serial determinations of red cell survival using Cr^{51}-labeled autologous cells have shown that increased survival time follows the induction of a remission whereas decreased survival time frequently can be correlated with progression in disease. This measure is too time-consuming to be of value as a routine method for the evaluation of chemotherapeutic agents. In selected instances, however, this approach can be used as an objective criterion for critical cancer chemotherapy evaluations.

The studies in man just described naturally led to an attempt to design a simpler test system in which tumors could be introduced at will and their degree of spread more easily controlled. A start in this direction has been made by a study of the effect of localized and disseminated Brown-Pearce tumor on the red cell survival of Cr^{51}-labeled rabbit erythrocytes. With each animal serving as its own control (having its normal RBC life span determined), various groups of animals were given Brown-Pearce carcinoma into the anterior chamber of the eye, the testes, or intravenously. The life span of the re-labeled red cells was then redetermined.

The results indicated that in those instances where the tumor progressed so rapidly as to kill the animal within a two to three week period there was no effect on the life span of the chromium-labeled erythrocyte. To allow progression of the tumor over a more prolonged time, multiple dilutions of tumor homogenates were administered intravenously. In these animals, although one of them survived as long as sixty-three days, there was no effect on the life span of the labeled red blood cell. In addition, another rabbit carcinoma, XV2, which has a life history of four to six weeks, was studied with analogous results. It was concluded from these experiments that in rabbits with two types of localized or widely disseminated carcinoma the erythrocyte life span was not affected by the presence of tumor.

Myeloma program

Studies in Dr. Elliott Osserman's laboratory have been directed along four major lines of investigation: 1) survey of the value of routine electrophoretic separation of the serum proteins in all cases of suspected or documented neoplastic disease; 2) diagnostic value of serum and urine electrophoresis in multiple myeloma; 3) serial electrophoretic studies of the serum and urine proteins in patients with myeloma as an objective criterion for evaluation of response to chemotherapy; 4) physico-chemical studies of myeloma proteins including isotopic studies of the rates of turnover of the serum and urine constituents.

In previous Scientific Reports of this Institute, the first two phases of the program have been summarized. The current status of work in the latter two areas will be presented here.

a) Serial electrophoretic studies of the serum and urine proteins in patients with myeloma as an objective criterion for evaluation of response to chemotherapy

Since all current studies support the thesis that the neoplastic myeloma cells produce the abnormal proteins which are demonstrable by electrophoresis, this biochemical abnormality represents a unique index in a neoplastic disease for the evaluation of chemotherapeutic effect. Employing the Durrum-type (Spinco) quantitative paper electrophoresis apparatus, we have followed the serum and urine electrophoretic patterns in 50 individuals with myeloma under a wide variety of therapeutic programs. In 2 instances, a profound decrease in the abnormal serum protein has been observed coincident with the administration of a specific chemotherapeutic agent. Concomitantly, there was ancillary subjective and objective evidence of suppression of the disease activity. In the remaining 48 cases, there has been no apparent decrease in the abnormal components and no other evidence of significant chemotherapeutic benefit.

b) Physico-chemical studies of myeloma proteins

Fundamental chemical studies of the abnormal serum and urine myeloma proteins have been carried out coincident with the clinical studies described above. Of particular interest has been the demonstration that the abnormal serum constituents when separated by filter paper electrophoresis show a marked affinity for Schiff staining after periodic acid oxidation. This is considered strong evidence in favor of the postulate that these serum constituents are conjugated glycoproteins, containing a significant amount of carbohydrate bound to the protein moiety. By contrast to this behavior of the serum constituents, the abnormal myeloma urinary proteins are "Schiff-negative". Accordingly, there appears to be a systematic chemical difference between the serum and urinary constituents when both are present in an individual case. It has also been observed that the abnormal urinary proteins in a given case of myeloma, exhibiting both serum and urine abnormalities, has a greater electrophoretic mobility than the corresponding serum constituent. Quite possibly, this difference in carbohydrate content may account for the observed differences in electrophoretic mobilities.

In order to explore further the interrelationship between the serum and urine constituents in a single case of myeloma, studies employing isotopically labeled aspartic acid were carried out in conjunction with Dr. Samuel Graff in the Department of Biochemistry. Ten grams of N^{15}-labeled aspartic acid was administered to a patient with myeloma, and the serum and urine proteins were collected and isolated over a period of two weeks. The dicarboxylic amino acids(glutamic and aspartic) were isolated from these two proteins, and their isotopic abundance measured. These studies revealed a marked difference in "turnover characteristics" between the serum and urine proteins. The serum constituent incorporated the label more slowly than the urine protein and showed a T 1/2 = 13 days. By contrast, the N^{15} label was rapidly incorporated into the urine protein, reaching a peak at 12 hours and thereafter rapidly declining, with a T 1/2 of 1.5 days. These results appear to indicate that the serum and urine proteins are independently elaborated, and that

the urinary constituent is not a fragment of the parent serum protein. We are still concerned, however, with the possibility that the serum protein may be unequally labeled in this type of an isotope study, and that a "superficial" portion of the serum protein molecule may receive a greater proportion of the labeled amino acid than the "central core". This "peripheral portion" of the serum protein molecule may "turn over" more rapidly than the "central core" and it is still possible that the protein found in the urine may represent this smaller molecular fragment. Further studies of this phenomenon are contemplated.

Biochemistry of Differentiation

Dr. Erich Hirschberg and Mr. Gerald Merson have continued experiments bearing on the biochemistry of differentiation in the slime mold Dictyostelium discoideum. The most intriguing feature of this organism, which motivated its inclusion in the general program outlined here, is the division of the life cycle into two distinct parts. In the first, the organism is unicellular and carries on active feeding and proliferation; in the second, it becomes multicellular and exhibits a complex series of morphogenetic movements in the virtual absence of cell division. This separation of the two principal phases of cell life, proliferation and differentiation, permits the investigation of biochemical events during the latter in the absence of synthesis of new protoplasm.

Interest has been focused principally on the first phase of differentiation in this organism, the aggregation of the single amoebae into the multicellular pseudoplasmodium. Three approaches are being employed: the inhibition of aggregation by various chemical agents of known mechanism of action and, when possible, the identification of normal metabolites which can block this inhibition; the comparative analysis of cells before and after aggregation in terms of various biochemical constituents; and an attempt to establish the identity of the chemotactic principle which is produced by the central cells and causes the peripheral cells to aggregate towards the center.

In an extended series of tests, it has been shown that aggregation is inhibited by low concentrations of a variety of agents which can be classed loosely as folic acid or pyrimidine antagonists (2,4-diaminopyrimidines, 1,2-dihydrotriazines, several miscellaneous antimalarials); citrovorum factor and, to a smaller degree, folic acid and thymine or uracil, block this inhibition. In general, the most effective inhibitors in a series are those which have the most complex sidechain substituents.

The chemotactic principle responsible for aggregation is present in very low concentrations in the cultures and is quite unstable. The results of current experiments are consistent with the assumption that it is a peptide containing at least four amino acids.

Neurosurgery

The Department of Neurological Surgery, under the direction of Dr. J. Lawrence Pool, continues its activities at the Francis Delafield Hospital, with special interest in (1) improved methods of effecting relief of pain; (2) studies in human neurophysiology, utilizing observations which may be obtained during the performance of pain-relieving surgical procedures; and (3) collaborative investigation with the Departments of Medicine and Radiology of various therapeutic methods applied to the treatment of malignant brain tumors.

Drs. Henry Liss and Thomas Bridges, in collaboration with the Department of Radiotherapy, are evaluating the effect of supra-voltage radiotherapy in a series of patients with malignant brain tumors.

Drs. Roger Baisas, Henry Liss, and John Potanos have collaborated with the Radioisotope Department in a study of the behavior of I^{131} when injected via the carotid artery in patients with known brain tumors.

Drs. Henry Liss, William Clark, and Thomas Bridges are preparing for publication a method of effecting pain relief in terminal carcinoma patients by "fractional" or "graded" saline-injection frontal lobotomy.

The technique of injection lobotomy is simple and relatively safe. Bi-frontal trephinations are made under local anesthesia. Forty-eight hours later a blunt-tip, lumbar puncture needle is inserted into one frontal lobe, anterior to the tip of the ventricle. Fifteen ml. of warm, physiological saline are injected in five ml. increments at each of three depths. This injection distends and destroys the white fiber bundles of the frontal lobe, producing a "lobotomy" effect with the immediate reduction of emotional tension and of pain. One to six injections at two to three day intervals may be required. Injections are continued only until satisfactory pain relief is obtained. Incontinence, stupor, and extremes of personality change are avoided.

Drs. Clark, M.D. Yahr, and Bridges have completed a series of studies of cerebral circulation based upon a plethysmographic method which they have devised.

This study has yielded these interesting observations:

1. Cerebral circulation responds to external stimuli, which activate the sympathetic, vasomotor system. The response is vasoconstriction.

2. The cerebral vasoconstriction response to cold or to pain is dependent upon the integrity of the cervical sympathetic chain.

3. Stimulation of the cervical sympathetic chain produces cerebral vasoconstriction, whereas stimulation of the stellate ganglion has no effect upon cerebral circulation.

4. Stellate ganglion procaine block results in diversion of blood from the cerebral circulation to that of the upper extremity.

The Departments of Urology and of Neurosurgery are continuing a study of hypophysectomy for advanced prostatic carcinoma. While excellent pain relief may be afforded in about 50% of cases of prostatic cancer, the effect of hypophysectomy upon progress of the disease and upon survival is yet to be determined.

Drs. Yahr and Bridges studied the digital vasomotor responses to dorsal and ventral nerve root stimulation during the performance of cordotomy for the relief of pain. They found that, whereas sudomotor outflow has been shown by Ray et al. to be limited to the second thoracic, and inferior thoracic, anterior roots, vasomotor fibers to the digital vessels transverse the lower cervical (C8) and thoracic one, two, and three roots. It is therefore necessary to speak of sudomotor and vasomotor divisions of the sympathetic nervous system, for the fibers subserving each function have differing pathways.

A study of the role of the dorsal root in vasomotor behavior revealed that an intact sensory pathway is required for preservation of normal segmental vasomotor tone. Sensory interruption is followed by a short period of vasoconstriction (paralysis of vasomotor function), and then by a period of vasoconstriction (unmodified vasomotor overactivity) which persists until sensation returns to normal. It is felt that temperature sense is the significant modality, and that disturbed vasomotor reactions result from impairment of temperature sensation.

Pathology

The Department of Pathology has continued investigations in the area of carcinogenesis, surgical pathology and histochemistry.

Drs. B.S. Oppenheimer, E.R. Oppenheimer, I. Danishefsky, and A.P. Stout have made further interesting observations on polymer-induced sarcomas in rats. The tumors have been produced by carefully prepared pure polymers as well as commercial products suggesting that it is not an impurity present in the latter that accounts for the tumorigenic action. In a series of convincing experiments, polymers were labeled with radiocarbon. Collection of expired air and excreta from animals bearing the C^{14}-labeled plastic led to the finding of radiocarbon after 21 weeks. This proves that the polymer is slowly metabolized and studies are now in progress to identify possible organic products.

Drs. L.W. Gorham and A.P. Stout have carefully studied eight cases of massive osteolysis. It was shown that this extraordinary disease which results in the complete or partial disappearance of one or more bones is associated with a proliferation of capillaries designated as a hemangiomatosis. It is thought that this is the mechanism of the bone resorption.

With Drs. O. Auerbach, T. Petrick, A.L. Statsinger, G.E. Muehsam, J.B. Forman, and J.B. Gere, Dr. A.P. Stout has been associated in a detailed study of the mucosa of the tracheobronchial tree. The study has consisted of step sections of the structures in non-smokers, light and heavy cigarette smokers and individuals with carcinoma of the bronchus. It was shown that basal hyperplasia, stratification and squamous metaplasia are increasingly frequent in the bronchial mucosa from the non-smokers to patients with invasive carcinoma of the bronchus. This contributes additional powerful evidence to the importance of cigarettes as a factor in the genesis of bronchogenic carcinoma.

Studies on the interdependence of cytoplasmic particulates by Drs. Silvio Fiala and Edith Sproul and Mrs. Anna Fiala with Corticotropin (ACTH) have shown that the growth of the adrenal gland induced by this hormone occurs in two stages.

The first is characterized by an increase of the cytoplasm with a striking formation of cytoplasmic, largely microsomal (chromidial) RNA and protein of the mitochondrial and microsomal fractions. The observations indicate that the mass increase of mitochondria is closely related to the increase in microsomal RNA. This effect is independent of cell division. In the second stage the mass, RNA and DNA content of the nucleus increases and cell divisions follow.

Another line of study by the same investigators has shown that a similar pattern of cytochemical action is true also for other hormones of the anterior pituitary gland such as the follicle stimulating hormones (FSH) and thyreotropin (TSH) in regard to corresponding target organs. In hypophysectomized animals practically no cell divisions occurred in distinction to intact animals, while the cytoplasmic growth induced by these hormones was even more pronounced. The further study of this growth promoting effect has shown that the increase of cytoplasmic RNA is preceded by a deep drop in the level of acid-soluble nucleotides within 1 - 2 hours after the injection of the hormones. Furthermore, it has been proven that the level of respiratory enzymes such as succinoxidase depends on the cytoplasmic RNA system contained in the microsomes. As long as the level of microsomal RNA remains unchanged following injection of the hormones there is no increase in succinoxidase.

It was of interest to compare in another line of investigation the growth promoting effect of tropic hormones with that of artificial carcinogens. It was found that carcinogenic azo dyes such as 3'-methyl-4-dimethylaminoazobenzene cause a slow increase in the ratio of the combined mass of nuclei plus the soluble phase of cytoplasm to the mass of cytoplasmic preformed elements (mitochondria and microsomes). This ratio corresponds to the slowly changing metabolic character of the liver cell following the feeding of the rat with the basal diet containing carcinogen. It was found that the cellular changes induced by azo dyes are in the opposite direction from the pattern of cytochemical action of pituitary hormones. A depletion of cytoplasmic RNA in microsomes precedes the depletion of the preformed elements (mitochondria and microsomes). The increase of the macromolecular ribonucleoprotein frac-

tion, the level of glycolytic enzymes and proliferation was observed only after gross depletion of cytoplasmic formed elements took place.

With the aid of Mr. Kenneth McCarty it was possible to follow the level of soluble ribonucleoproteins obtained by using the Spinco ultracentrifuge on the adrenal and liver homogenates and to correlate it with the level of glycolytic enzymes during carcinogenesis and after hormone administration. These preliminary experiments suggest strongly that just as the level of respiratory enzymes is dependent on preformed cytoplasmic RNA so is the level of glycolytic enzymes dependent on the level of soluble ribonucleoprotein fraction.

Dr. Fiala, with the assistance of Mrs. Fiala, has been attempting the isolation of the liver fraction which firmly binds the carcinogenic azo dye. This fraction was found to be mainly a peculiar albumin. Preparations several thousand times purer (on the basis of nitrogen) were recently obtained. This work is being continued in an expanded form.

Dr. Fiala and Mrs. Fiala determined quantitatively the fractions obtained by differential centrifugation from Ehrlich ascites tumor cells. Cytochemical and metabolic characteristics, expressed on the cellular basis of this tumor, are in complete conformity with the conclusions reached before, namely, that in malignant cells the mass of cytoplasmic formed elements is low when compared with the combined mass of nucleus plus the soluble phase of the cytoplasm. The DNA content of the average nucleus of the Ehrlich ascites tumor cell was found to be $12.4 - 13.8 \times 10^{-12}$ g, suggesting high degree of polyploidy.

Radiological Research Laboratory

As in the past, the activities of the Radiological Research Laboratory have been divided between a research project for the Atomic Energy Commission and problems related to therapeutic radiology and medical uses of radioactive isotopes. While the work carried out for the AEC is concerned with problems of special interest to the Commission, it generally involves fundamental problems in radiological physics and radiobiology.

The radiological physics section is primarily concerned with absolute measurements of both the quantity and quality of radiation as delivered to tissue. In addition to investigating new methods of such measurements, considerable effort is being spent in determining certain parameters which are essential in the precise dose measurement involving the ionization method. Radiobiology under the Atomic Energy Commission contract has been limited to specialized problems which have bearing on radiation protection and which may shed some light on the biological action of radiation.

Radiological Physics (Drs. Failla and Rossi, Mr. Gross, and associates.)

Dose determinations involving tissue equivalent ionization chambers have been performed at several installations in order to provide an absolute calibration of exposures in radiobiological experiments. These measurements involved both fast and slow neutrons. Further progress has been made in the development of an instrument for the measurement of dose as a function of LET (Linear Energy Transfer). The instrument has been used in the evaluation of various spectra and this work has demonstrated certain limitations in the LET concept.

Studies utilizing chambers having internal gas multiplications have indicated that such chambers may be operated with multiplications of at least as high as 10^4 without any deviations from linearity over intensity variations of the order of 10^3.

A calibration of a strontium-90 surface applicator was performed. A method for the determination of the absolute emission rate of charged particles which has been developed over the last five years has been tested. Both relative and absolute de-

terminations of W (average energy loss per ion pair produced) for electrons were carried out, and a determination of W for water vapor is in progress.

A number of measurements were also performed on relative specific energy absorption in various media. The study disclosed a satisfactory correlation between theory and experiment. The method of measurement of K-capture isotopes which was described in last year's report has been further developed. Checks showed good agreement with other techniques.

Further research has been done on determining conditions required for complete saturation of ionization chambers.

The measurement of tissue dose produced by isotopes localized in small lesions has yielded the point source ionization function of P^{32} and S^{35} at distances near the source.

The character of health monitoring instruments when used to determine a dose delivered by plane thin beta source has been investigated, yielding a number of practically useful correction factors which may be applied in routine measurements.

A number of ancillary problems investigated include a study of the characteristics of radiophotoluminescent dosimeters. A special high sensitive photo-cell circuit has been adapted for the purpose.

Experiments by Dr. Ruth Hill utilizing a phage T_1 and a newly discovered mutant, T_{1s}, have shed some light on a mechanism of spontaneous as well as radiation-induced mutations in bacteria.

Radiobiology (Drs. Roberts Rugh, Z. Dische, Mr. Allen, and associates.)

Studies based on young and adult rats and mice have been continued. The retina of the fetal mouse between 13.5 and 15.5 days has been found to be seriously damaged by as little as 50 roentgens, the damage at 4 hours consisting of pycnotic and karorrhectic nuclei. By 24 hours giant macrophages (phagocytes) begin to clean away the dead cells and by 72 hours the retina appears to be reconstituted, not by recovery of damaged cells, but by replacement from undamaged and primitive neurectoderm

cells. The developing brain also reveals areas of active differentiation if exposed to x-rays in doses from 100 to 300 r.

Radioiodine uptake in the thyroid gland of the pregnant mouse can be reduced by the administration of potassium thiocyanate either before, during, or even 4 hours after the injection of radioactive iodine. The thiocyanate protects both the maternal gland and those of her fetuses. Female mice were given single or fractionated doses of 50 to 300 r, and subsequently tested for fertility. Complete sterilization of all females occurred by 8 weeks following 100 r, and was in some cases achieved by as little as 30 r given at the rate of 10 r per week for three weeks.

The rate of reaction and recovery of adult vs. weanling rat peripheral blood (males and females) following acute (600 r) whole body x-irradiation was studied over an extended period of 175 days. Current research deals with determination of the minimal dose of x-rays which will produce sterilization in adult and fetal mice; effect of β-mercaptoethylamine and becaptan disulphide on ovarian sterilization by x-rays; relation of gonad hormones to radiation-protection, particularly in castrated males, and effects of irradiation in utero on psychological behavior in rats.

A study of the protein metabolism of the lenses of both young and adult rats, controls and irradiated, did not show appreciable differences in the tryptophan content even though it is known that young animals fed on tryptophan deficient diets tend to get cataracts in their lenses.

A further study of the post-irradiation protection of rabbits by the intra-peritoneal injection of splenic plasma shows that survival of the treated group was significantly (at the 0.01 level) greater than irradiated but untreated controls. Rabbits treated with dog splenic plasma were also afforded significant protection.

Clinical radiation physics (Dr. Rossi, Mr. De Friess, and associates.)

Most of the work has been centered around the betatron. The installation of the machine was completed on March 30th and the first patient was treated on September 20th. In the intervening period a considerable amount of work had to be done to insure constancy of operation, proper alignment, and accurate dosimetry. Some of

this work is still continuing. In particular, it has been found that various components of the machine have to be modified slightly in order to attain a greater degree of reproducibility. A number of accessory pieces of equipment had to be built. These include a treatment table designed by Dr. Failla, various monitoring ionization chambers, treatment cones, and absorbers used in special treatment procedures. Problems which have been pursued in particular involve a number of depth dose measurements, both near the skin surface and at greater depth in phantoms. Some work has been done on trying to develop better techniques for obtaining localization films. However, the results obtained are not adequate and further work will have to be done along these lines in the near future. An intercomparison has been performed involving similar betatrons at the M.D. Anderson Hospital in Texas, Mallinckrodt Institute in St. Louis, and Memorial Hospital. The results make it possible to compare the relative magnitudes of the dose units employed at these institutions. Work is in progress to determine the absolute dose delivered per unit. Initial experiments have yielded this figure to an accuracy of the order of 10%. It is planned to obtain a figure of higher precision as a result of the projected work.

Other problems of radiological physics have been largely matters of routine, including calibration, treatment planning, and a considerable number of protection studies. Special dosimetric problems include measurements with a recently acquired recording instrument which can be used to determine dosage in body cavities. The results of these measurements have been compared with dose computations based on orthographic x-ray projections of radium implants using the method developed by Kligerman et al. This work is being done in cooperation with Dr. S. Gusberg of the Department of Obstetrics and Gynecology.

A technique has been developed for the fabrication of molds containing a beta-emitting isotope (Y^{90}). These molds are used for the treatment of superficial malignancies, and a method has been found of determining the dose delivered accurately.

Cancer research project on radiation dosage (Dr. E. Quimby and Mrs. Cohen.)

Work has continued on methods to improve determination of dose delivered at a specific region in the body. During the past year detailed tables and procedures have been prepared for the calculation of isodose charts on a basis of currently available depth dose data. Calculated charts are in excellent agreement with those determined experimentally.

A study is currently being carried out on the effects of radiation quality, target-axis distance, and field size on the efficiency of rotation therapy of lesions in different parts of the body.

Radiology

Presbyterian Hospital

With a grant from the United States Public Health Service a 24 million volt betatron was installed and tested in 1955. However, the cost of reconstructing an existing high voltage treatment building which was more than half again the cost of the equipment has not been met. The betatron project is a long-term one in cancer research.

The betatron is a high energy x-ray machine for the treatment of deep-seated and recurrent cancer and allied diseases. Many physical measurements and animal experiments were undertaken to insure safety in the treatment of patients. The first betatron patient, who had a recurrent malignant tumor of the skull, was treated on September 20th and was televised nationally on "The March of Medicine." Since its opening, betatron operating time has been divided between clinical treatments for malignant diseases and physical measurements on a research basis. Research must be carried on continuously to improve conditions under which cancer patients are exposed to supervoltage radiation.

Dr. Harold Jacox continued his studies on the results of radiotherapy in carcinomas of the breast, lung and uterus. He is working on a clinical study of the results of the radiation treatment of cancer of the urethra.

Dr. Russell Wigh in association with Dr. Norah duV. Tapley made studies on the roentgen characteristics of cancer metastatic to the large bowel.

Dr. Jerome Nadelhaft is continuing a study of all salivary gland tumors treated at Presbyterian Hospital.

Dr. Juan Taveras is working with Dr. Lester Mount on the evaluation of radiation therapy of gliomas of the optic nerve and chiasm. With Dr. Charles Posner he is working on cerebral angiography in encephalo-trigeminal angiomatosis, and with Dr. Francis Cummins and Dr. Edward Schlesinger, he is determining the value of radiation therapy in gliomas of the third ventricle and pinealomas. Dr. Taveras is collaborating with Drs. Nadelhaft and Paul Wermer on a study of unusual roentgen manifestations

of reticuloendotheliosis. He has been evaluating the shift of the midline structures in cerebral angiograms and the forward displacement of abdominal viscera by retroperitoneal masses and tumors. He has been investigating the roentgen findings in cranial and cerebral trauma in infants and children and the angiographic diagnostic features of intracranial neoplasms by abnormal tumor circulation. Dr. Taveras is working with Drs. Schlesinger, Harald Rossi, and Edith Quimby on the development of improved techniques in radioactive isotopic localization of brain tumors.

Dr. Donald Barnett is working on a clinical study of craniopharyngioma. The Electrogastrographic Group under the direction of Dr. Edmund Goodman is now obtaining a radiographic record of the position of the gastric electrodes during each test. The Department of Radiology is cooperating in differentiating gastric cancer from other conditions discovered by radiologic means.

Dr. Gerhart Schwarz in collaboration with Dr. Donald Johnson is making a statistical study to test the validity of a new roentgen sign hoped to permit a reliable differentiation between benign and malignant gastric ulcers.

Dr. Josephine Wells completed a study of calcified liver metastases and is starting a survey of radiation induced leukemia. Drs. Catherine Dalton and Samuel Madell are working on roentgen detection of cancer of the pancreas.

Dr. Morton Kligerman with Drs. Quimby, Madell, and David Habif, made a statistical appraisal of the use of radioactive iodinated human serum albumin for the detection of liver metastases. He is conducting an experiment with Dr. Daniel Shapiro on the augmentation of radiotherapeutic effects by multicombination cancer chemotherapy. In conjunction with Drs. Algernon Reese, Tapley, and George Hyman, Dr. Kligerman is conducting a controlled study on the treatment of retinoblastoma by radiation alone and by triethylenemelamine and irradiation. He is continuing research with Dr. Virginia Frantz on a study of carcinogenesis from external and internal radiation in the thyroid of the Long-Evans rat.

Francis Delafield Hospital

A. Radiotherapy

Dr. Ruth Guttmann is still concentrating on the evaluation of supervoltage irradiation, from the clinical as well as the physical point of view. A number of publications summarizing these results are included in the bibliography of this Report.

The Physics Laboratory of the Delafield Hospital carried out the following studies:

1) Transit dose measurements for cobalt 60 rotating teletherapy by Drs. Carl B. Braestrup, George Hertsch, and Richard T. Mooney.

The object of this study is to determine the transit dose rate during moving beam therapy and to obtain a graphical representation on polar coordinates of the exposure rate.

A pilot model of the system has been completed and is used routinely on phantoms. Measurements on patients have just been initiated. The system promises to be of real value in determining the actual dose delivered to the tumor by indicating the correction factors which have to be applied to phantom isodose measurements due to the difference in density between tissue and water or masonite. It should be useful also in positioning of patients and in duplicating techniques. Much experimental work remains still to be done before its accuracy and maximum clinic value will be established.

2) Attenuation measurements of scattered cobalt 60 radiation in lead and building materials by Drs. Carl B. Baestrup and R.T. Mooney.

The purpose of this investigation is to study the shielding requirements against cobalt 60 scattered radiation.

Extensive data have been obtained on the attenuation of cobalt 60 gamma radiation scattered at various angles. The results are now being worked up in a form suitable for protection design.

3) Isodose patterns for moving beam therapy by Drs. Richard Mooney, Eleanor Englemann and Carl B. Braestrup.

Isodose studies have been made using photographic films with the aim of providing the radiotherapist with standard isodose patterns for different rotational techniques.

B. Radiodiagnosis

Dr. Arnold Bachman reports the following general research objectives and the status of the research:

1) Local angiography in neoplastic cases to distinguish between benign and malignant tumors and to determine operability.

The injection of veins and arteries near tumors may demonstrate pressure on or infiltration of these vessels by the tumors. This is of aid in differentiating benign and malignant lesions. Pulmonary angiography is particularly helpful in demonstrating obstructive or infiltrating lesions in mediastinal vessels and thereby is useful in evaluating metastases otherwise invisible.

2) Intra-osseous angiography, particularly with respect to ribs and vertebrae, is being performed in an attempt to determine bony, mediastinal and paravertebral metastases.

Following intra-osseous injection into the ribs and vertebrae the intercostal and paravertebral veins are quickly delineated radiographically. The intercostal vein filling also leads to good demonstration of the azygous venous system in the mediastinum. Obstruction and infiltration of the azygous vein almost always indicate inoperable invasion of the mediastinum in cases of carcinoma of the lung.

3) Investigation of the radiographic appearance of post-radiation fibrosis of the lung following radiotherapy in cancer of the breast is well advanced. This work is now being done in collaboration by Dr. Doris Bate with Dr. Guttmann.

4) An investigation of the value of a new intravenous biliary contrast material, cholografin, in association with Dr. C. Crandall of the Department of Medicine, which was begun last year, is now being completed. The investigation has shown that doses considerably larger than those advocated are entirely safe, but do not yield routinely better visualized biliary ducts.

5) Certain aspects of the radiographic appearance of the larynx have been studied by Drs. Bachman, Bate , and Oscar Ruiz. The radiographic appearance of submandibular lymph nodes, the pre-epiglottic space, the epiglottico-ventricular angle and the disassociation of laryngeal ventricles has been given special study.

C. Radiobiology

Dr. Anna Goldfeder made comparative studies on the biologic properties of two animal tumors indigenous to the same host, and investigated the influence of the time factor in radiation effects on biologic systems. She is continuing an attempt to establish a relationship between rate of growth, metabolic activity and radiosensitivity of various types of tumors, and is carrying out experiments to establish sensitivity and metabolic activity of two types of tumors growing in parent hosts, i.e., in the hosts from which they originated.

Surgery

Drs. Edmund N. Goodman and Henry Colcher of the Department of Medicine, and their associates have continued their experimental and statistical investigation of the electrogastrogram in patients with gastric or duodenal abnormalities. The apparatus permitting the placement of five electrodes in the stomach has been developed further and preliminary records support the belief that this will prove to be a fruitful field of investigation. A group of patients with non-malignant gastric abnormalities have been studied repeatedly in order to establish the consistency of electrogastrographic records as well as to correlate changes with the clinical behavior of the patient. Studies are continued on patients with suspected or proven carcinoma of the stomach. Localization of the electrodes is plotted by x-ray films exposed during the study.

Of special interest was the participation of Dr. Goodman's group in a symposium on the electrogastrogram. The material presented at this conference has been published in pamphlet form.

Dr. Harold Harvey has continued his statistical evaluation of the post-operative results in patients suffering from carcinoma of the stomach. Through long term accumulation of such data it is hoped that further improvements in surgical techniques may be developed, as well as a better appreciation of the natural history of this condition.

Drs. Robert Grinnell and N. Lane continue their study of adenomatous polyps and papillary adenomas of the rectum and colon. Of special interest during the past year have been the clinical and pathological aspects of recurrences of adenocarcinoma of the colon and rectum at the suture line following surgical resection. In addition, an analysis of squamous cell carcinoma of the anus was undertaken.

Drs. Frederic Herter and Thomas Santulli have conducted an experimental evaluation of the influence of bacteria in the intestinal tract on the implantation of tumor cells at the suture line following resection for carcinoma. This work was undertaken because of 1) the relatively high incidence of local recurrence of car-

cinoma at the suture line, 2) circumstantial evidence that these anastomotic recurrences are due to direct implantation of viable tumor cells in the lumen of the colon at the time of resection, 3) an experimental study by Vink who concluded on the basis of a small series of experiments in rabbits that the normal bacterial flora in the intestine acted as a strong deterrent to the implantation of tumor cells at the suture line. The current study is employing suspension of Brown-Pearce carcinoma cells obtained from tumors transplanted to the eyes of rabbits. As a control study these tumor cells were injected free into the peritoneal cavity to demonstrate consistent positive growth after transplantation. Other preliminary studies involved a bacteriologic investigation of the intestinal flora of rabbits. It was found that oral neomycin given for 24 hour periods could reproduce with accuracy the pattern achieved in humans after antibiotic preparation of the intestine. Work is in progress with the implantation of these tumor suspensions into an isolated segment of ascending colon of the rabbit at the time of resection of a small segment of colon and end-to-end anastomosis.

Dr. Daniel M. Shapiro, together with Dr. M. Shils, and Dr. L.S. Dietrich of the Department of Biochemistry have continued their studies exploring the rationale of employing quantitative biochemical differences between neoplastic and normal tissues as a basis for cancer chemotherapy. To date, five vitamins (thiamine, riboflavin, pyridoxine, niacin, and coenzyme A) have been documented as being in low concentration in a mouse mammary adenocarcinoma (755). Two new niacin antimetabolites have been studied and revealed provocative results in that adding these compounds to a previously described triple combination (azaguanine plus deoxypyridoxine plus testosterone) yielded complete cessation of tumor growth.

Still more encouraging has been the demonstration that another multi-combination can produce tumor regression. These latter findings can only be produced by the complete multi-combination, i.e., no lesser combination of these same compounds is capable of producing regression no matter how high the individual dosage of each compound is pushed. These findings are considered highly encouraging toward the

over-all goal of this program, namely, that multi-combination therapy is capable of producing metabolic damage at sufficiently diverse points within the cell so as to summate in lethal damage. The data is interpreted by the investigators as establishing one of the basic principles of the chemotherapy approach.

Studies to determine the biochemical locus at which anti-tumor drugs exert their effect have continued. Omega-methyl pantethine and bis (pantoylaminoethyl) disulfide, two pantetheine analogues, were shown to have strikingly different affinities in vitro for two coenzyme A dependent systems, transacetylase and citrogenase. Omega-methyl pantethine is a very effective inhibitor of transacetylase but has little activity against citrogenase. Bis (pantoylaminoethyl) disulfide, on the other hand, is an effective inhibitor of citrogenase having little activity against transacetylase. This latter compound also inhibits the in vitro synthesis if co-enzyme A from pantetheine ATP.

It was observed that hydroxylamine was an inhibitor of endogenous uric acid formation. It was subsequently shown that of the enzymes concerned hydroxylamine specifically inhibited xanthine-oxidase activity. It was further determined that hydroxylamine reacted with the riboflavin portion of the FAD co-factor in a manner to prevent normal oxidation of the isoalloxazine nucleus.

Collaboration with Dr. M. Kligerman of the Department of Radiology on a combination attack of multi-combination chemotherapy plus radiotherapy was evaluated on a mouse mammary adenocarcinoma. It was necessary first to establish a regimen of radiotherapy which did not destroy the tumor but did cause slight damage. The results of experiments employing this radiation dosage, together with multi-combination chemotherapy, demonstrate a greater cure rate with the over-all combination than is obtainable with either modality of therapy alone. Further studies employing a more powerful multi-combination of antimetabolites together with radiation are currently in progress.

Drs. Cushman D. Haagensen and Dan H. Moore have continued investigation of the milk agent in mammary carcinoma of the mouse. During the past year the results of

experiments designed to determine the extent of activity of the milk agent have been completed. These experiments show that the milk agent can indeed be titrated and that whole milk is still active at dilutions of 10^{-10} but dilutions of 10^{-12} show little or no activity. The results of these experiments now make it possible to study the virus in a quantitative way. These experiments also show that the milk can be lyophilized with little loss in its ability to produce cancer.

In collaboration with Dr. Ernest Pollard at Yale University the cyclotron is being used to irradiate milk samples so that the size of the infective agent may be determined. The results of these experiments when completed within about two years should indicate whether the milk agent is actually a virus.

In collaboration with Dr. Margaret Murray, pure strains of mammary epithelial cells are being grown in tissue culture for the first time. This is done in order to study the effect of the milk agent on normal mammary gland cells when inoculated into the test tubes with the growing tissue. If it should be possible to convert normal cells into cancer cells, the whole process of this cancer etiology would lend itself to direct and detailed study.

Dr. Hugh Auchincloss, Jr. has analyzed a group of about 150 patients with mammary carcinoma and having mastectomies by the dermal dissection technique. Specific interest in the follow-up ranging up to 10 years has been directed mainly at the percentage of local recurrences as compared to those following other techniques of mastectomy.

He has in process a study of more than 30 patients with positive internal mammary nodes secondary to carcinoma of the breast. These patients had radical mastectomies with partial resection of chest wall (Urban procedure). The follow-up of this group is still under five years.

Surgical pathology

Dr. Carl R. Feind has conducted a study of head and neck surgical specimens. The Surgical Pathology Department has cooperated in clearing these specimens in the hope of adding to the knowledge of lymphatic spread of cancer of the head and neck.

To date much information has been accumulated which might ultimately help the surgeon determine the adequacy of his excision as well as the need for more far reaching radical surgery.

Dr. R. Lattes completed a statistical study of 80 cases of thymoma.

Dr. Virginia K. Frantz and her associates have continued the study of thyroid cancer, with detailed study of specimens from radical dissections in an effort to document routes of spread of the disease, and with serial sections to establish possible histological variations in primary tumors and their metastases.

They have almost completed a study on carcinogenesis in the thyroid of the Long-Evans rat from internal and external radiation.

Drs. Haagensen, Lattes and Horsley have reviewed 86 cases of Paget's disease of the breast. It was shown that in all cases in which histological study was adequate, the Paget's changes in the skin were associated with intraductal carcinoma of the lactiferous duct. In almost all cases there was also intraductal carcinoma deeper in the breast.

Dr. John Pickren, now Chief Cancer Research Pathologist at the Roswell Park Memorial Institute in Buffalo, has continued but not completed his study of serial sections of axillary lymph nodes from cases of cancer of the breast. This study aims at determining the actual incidence of microscopic metastases in lymph nodes reported negative on routine sections.

Dr. Nathan Lane has completed a similar study on serial sections of regional lymph nodes from ten cases of malignant melanomas. These nodes had been previously reported negative on routine sections; three of these contained microscopic nests of cells interpreted as malignant. This is part of a project of clinical-pathological study on their series of malignant melanomas conducted by Drs. J. Malm and R. Lattes.

The Histochemistry Research Laboratory, under the direction of Dr. Gabriel C. Godman, has continued investigations on 1) nuclear constituents in the course of cell differentiation and division, in viral infection and in other morbid states, and 2) chemical differentiation of cells of mesodermal origin.

In collaboration with Dr. David P. Bloch it has been shown using photometric methods that in morphologically indistinguishable interphase nuclei those which are functionally differentiated may be distinguished from those in preparation for division, owing to distinct differences in the state of their deoxyribonucleoprotein complexes. In connection with studies on the synthesis of DNA and nuclear protein in the cell cycle, Dr. Bloch has proposed a plausible mechanism for the replication of new helical strands of the DNA molecule based on the molecular model of DNA-protamine. He has suggested that interruption of the hydrogen bonds of the bases takes place before replication, enabling the bases to rotate 180° about their glycoside bonds in the single polynucleotide strands held in position by basic protein. This should make possible alignment of new nucleotides by attachment through their complementary bases, and the formation of a replicate chain by internucleotide links.

The deoxyribonucleoprotein changes in various pathological conditions are under study. The course of intranuclear viral infection with types 3, 4, and 7 of the RI (APC) human virus has been the subject of a correlated electron-microscopic and histochemical investigation in association with Drs. C. Morgan, C. Howe and H. Rose of the Department of Microbiology. The crystalline intranuclear aggregates of viral particles were found to consist of a nonhistone deoxyribonucleoprotein with properties different from those of the intrinsic nuclear chromatin. A method of differential staining of viral and host DNA based on differences in relationship with their associated protein moieties has been devised. The viral DNA was found to develop from a matrix which does not contain DNA. Quantitative studies of the effects on DNA synthesis of the virus responsible for human skin papilloma have also been extended to indicate that the markedly accelerated intranuclear DNA synthesis does not occur in stages which increase in regular multiples. In the later stages of intracellular infection, all of the DNA is localized in the inclusion and is presumably viral. Infected cells do not divide. Uninfected cells of the hyperplastic zone show a distribution of DNA content like that of the normally active stratum mucosum.

The marked alterations of nuclear contents occurring in leucocytes in lupus erythematosus were also studied microphotometrically with Dr. A.D. Deitch. It was found that depolymerization of DNA in these bodies could not be detected, and that observed changes in dye binding could be related to the association of DNA of nuclei affected by the lupus factor with unusual protein.

Investigations of the effects of hydrocortisone on nucleoprotein formation by cells in vitro, in collaboration with Dr. H. Grossfeld of the Department of Medicine, have also been started.

Work on the production of acid mucopolysaccharide by cells in large scale tissue cultures has been continued in collaboration with Drs. H. Grossfeld, K. Meyer, and C. Bassett, and Mr. C. Morris. Mass cultures of additional types of mesodermal tissues are being analyzed. A sensitive and accurate method of evaluated growth and cell numbers in cell culture preparations from which such data were heretofore difficult or impossible to obtain, has been elaborated by Mr. C. Morris in collaboration with Dr. T.D. Price of the Department of Biochemistry, using the ultraviolet photometric-densitometric method for estimating DNA in chromatographic spots on paper. Information derived from this method is essential to the conduct of experiments on the relationship between growth and mucopolysaccharide formation.

The overall purpose of the Laboratory for Cell Physiology, under the direction of Dr. Margaret Murray, is to utilize the methods of tissue culture to investigate a variety of medical problems at the cellular level. Progress has been made in several areas, particularly that involving the nervous tissues.

Dr. Arlene Deitch (Post-Doctoral Fellow of the National Institute for Neurological Diseases and Blindness) has completed a cytological study of developing spinal ganglion cells in which she has shown that discrete Nissl bodies are not to be considered artifacts of fixation, but pre-exist in the living cell. Crucial data on this point have been obtained by making successive phase-contrast photographs of the same cell 1) living, 2) fixed, 3) stained with basophil dyes for Nissl substance. Ultra-violet absorption studies of the living cell, undertaken with Dr. Montrose

Moses of the Rockefeller Institute, corroborate the data obtained by phase-contrast microscopy.

Following up her earlier studies on myelin production in vitro and circumstances surrounding it, Mrs. Edith Peterson has been investigating the nutrition of the spinal ganglion complex with the aid of chemically defined media. Parker and Healy's 858, which is adequate for growth and maintenance of fibroblasts, is not adequate for these ganglion explants, in which we are dealing with a mixed population of cells whose mutual contributions to the progressive differentiation of the whole must be maintained in delicate balance. In the synthetic medium, unsupplemented, the Nissl pattern of the neuron may mature normally, but Schwann and satellite cells are adversely affected; reticulin and myelin sheaths are not produced. It appears that the neuron may produce and store nucleic acids visualized as Nissl substance in the absence of normally functioning satellite and Schwann cells, but is not able to form myelin and reticulin sheaths without their participation.

Pursuant to his pioneer work in recording action potentials from spinal ganglion cells in vitro, in which both cell and penetrating electrode are visualized under the high-power microscope, Dr. Stanley Crain has been extending his techniques to deal with less favorable, but in some respects more desirable neuronal material. Action potentials in response to electrical stimuli have now been obtained from cultures of chick embryo spinal cord and sympathetic ganglia, demonstrating that function can be maintained in these tissues also for at least several weeks in vitro. Human brain and sympathetic ganglia have so far resisted his efforts to register electrical activity, and he is attempting to identify the factors responsible for this divergence in behavior.

Mrs. Helena Benitez has prepared a brief time-lapse movie film demonstrating in vitro the tetanic response of oligodendroglia to serotonin. She has shown also that in these cells (from rat and human brain) there is an interaction between the effects of serotonin and those of lysergic acid diethyl amide. These observations reveal a mechanism by which such drugs may exert direct physiological effects upon brain cells.

In the course of studies on the Bittner milk factor and its relation to mouse mammary carcinoma, Dr. Etienne Lasfargues has developed a method of growing normal adult mammary epithelium in quantity in continuous culture. This is based on the dissociation of the mammary tissues with collagenase and subsequent separation, by centrifugation, of the cellular from the fibrous and lipid components. This long-desired achievement will be of value also in other fields, e.g., endocrinology and human cancer. Its immediate application has provided continuous cultures of adult mouse mammary epithelium which can be inoculated with the milk factor and assayed periodically as a substrate for the multiplication of the agent. Pilot experiments are under way to investigate the possibility of detecting the milk factor in tissue culture by means of fluorescent antibodies. Success in this direction would reduce the bio-assay period from about 2 years (in vivo), to 10 days, in vitro.

In the field of cancer chemotherapy, Mrs. Ruth Eising is cooperating with Drs. Gellhorn and Hirschberg and members of the Neurosurgical Service. This work involves a 4-way correlation between the effects of a series of carcinolytic compounds on a transmissible mouse glioma in vitro and in vivo and the human glioblastoma multiforme in tissue culture and in clinical trial. Encouraging results have been obtained with the anti-malarial quinacrine, which is lethal to the mouse and human tumors at low concentrations (M/20,000) in vitro. However, though it is possible to get this compound distributed into human and mouse plasma at these levels, it appears that the concentration of the drug in the tumor cells in situ reaches only 1/10 - 1/5 this amount. Related compounds are under study.

In collaboration with Drs. Arthur Purdy Stout and Raffaele Lattes, Dr. Murray has continued her efforts to extend, through observations in vitro, the existing criteria for classifying and diagnosing human neoplasms.

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During the past year Dr. Grant Sanger has made a statistical review of all the colon and esophagus carcinomas that have been seen in Delafield since its opening, and with Dr. Whitney Woodruff all of the cases of breast carcinoma have likewise been reviewed and analyzed.

Experimental work on the palliative relief of ascites by means of exposure of the mucosal surface of the terminal ileum within the peritoneal cavity, as first suggested by Neuman and associates (Surgical Forum of American College of Surgeons, 1955), has been carried on by Drs. Frederic P. Herter and Archibald G. Fletcher, Jr. Experimental ascites is produced in dogs by constriction of the inferior vena cava above the diaphragm. A segment of terminal ileum is then isolated and the mucosal surface everted within the peritoneal cavity as an absorbing surface. The results so far have been equivocal. The method does not appear promising for the relief of ascites due to portal cirrhosis, but it may prove of value in the palliation of patients with persistent and incapacitating ascites due to peritoneal implants of carcinoma.

On the Head and Neck Service at Delafield Hospital, Dr. Varham Bakamjian has continued the work in the development of plastic techniques for reconstruction of surgical defects following extensive excision for cancer in this area.

Urology

Under the direction of Dr. Perry B. Hudson, the group which includes Drs. T.D. Price, Michael E. Lombardo, and Morris London have continued their research activity on the subject of genito-urinary cancer along several lines. These include investigations in steroid chemistry, enzymology and nucleic acid metabolism, and have in part utilized clinical material from the urology ward of the Francis Delafield Hospital. In the interest of clarity, the progress made in these three categories will be reported separately.

In the field of steroid hormonal chemistry, studies have been undertaken to elucidate the nature of the steroid hormones in human testicular vein blood and adrenal vein blood obtained from patients with disseminated carcinoma; to establish the quantitative nature of urinary estrogens in female patients with metastatic mammary cancer before and after ovariectomy and total adrenalectomy; to isolate and identify the metabolites of cortisone and meticorten from the urine of adrenalectomized patients maintained on these substances; to study the role of the kidney in the metabolism and excretion of steroid substances; and to study the pathways in the biosynthesis of the adrenocortical hormones by the human adrenal and of the testicular hormones by the human testis.

Testicular vein blood, peripheral vein blood and adrenal vein blood have been collected from a number of patients, extracted and fractionated, and are currently being subjected to chromatographic separation and identification. Preliminary results from a detailed study on urinary estrogen excretion in female patients with metastatic mammary carcinoma before and after ovariectomy and total adrenalectomy show a definite trend toward a depletion of urinary estrogens following total adrenalectomy. In collaboration with Dr. Charles Crandall of the Department of Medicine, corollary clinical studies were made of the calcium metabolism of mammary cancer patients with osteolytic metastases to determine possible preoperative criteria to foretell beneficial results from adrenalectomy. However, the results were inconclusive.

The metabolites of cortisone in the urine of an adrenalectomized patient have been isolated and identified. In in vitro perfusion experiments on functional dog kidney preparations with six steroids of varying polarity, it was found after fractionation, extraction, separation and purification that 9 to 12% of cortisone and hydrocortisone and 2.9% of Reichstein's compound S were recovered in the free fraction of the urine, whereas only microgram quantities of the less polar steroids were recovered. No metabolites of the perfused steroids could be isolated in any of the perfusions with the exception of testosterone, which was converted to Δ^4-androstene-3,17-dione, and in none of the experiments were any steroids found in the sulfate or glucuronide fractions of the urine. In an in vitro study of adrenal glands obtained from female patients with metastatic carcinoma of the breast, the conversion of progesterone, 17-α-hydroxyprogesterone and 11-deoxycortisol to cortisol was demonstrated.

Several enzymes are being studied - prostatic acid phosphatase to determine its kinetic properties and mechanism of denaturation; uricase as a potential means of lowering uric acid levels in cancer patients; and tripeptidase to establish its properties, develop an assay procedure and evaluate the significance of elevated levels in the sera of cancer patients.

A number of inhibitors of prostatic acid phosphatase have been identified and classified, and a theory developed to explain the mechanism of their behavior. A purified form of uricase has been administered to patients, with a resultant temporary excretion of allantoin and decrease in serum uric acid levels. Assay and isolation procedures for tripeptidase have been developed, and studies are under way to determine a possible correlation between cancer processes and serum tripeptidase levels.

With regard to nucleic acids, research is being undertaken to obtain information on the biosynthesis and the physiological and pharmacological factors capable of influencing the nature and rate of formation of these substances in human tissues, and to elucidate aberrations in their metabolism known to exist in human cancer cells.

A substantial amount of information has been gained on the rate of renewal of individual chemical components of nucleic acids obtained from the human adrenal, kidney, ovary, prostate and testis, and the low weight nucleotides are also being studied. Investigations on the permeability of the cells to nucleotides have revealed that the cell membranes are readily penetrated by nucleosides and phosphate, which combine to form nucleotides. Tracer studies with radioactive phosphate have shown that rates of renewal of phosphorus of intracellular "small-molecule" nucleotides, ribonucleic acids and deoxyribonucleic acids in human organs have relative values approximating 10,000, 100, and 1, respectively. Other studies on the mechanism of nucleic acid biosynthesis have been carried out on mice and bacteria, the latter in collaboration with Dr. S. Zamenhof of the Department of Biochemistry.

The statistical group has been increased in numbers to continue amassing statistical data concerning the incidence of prostatic cancer in the aging male population, the results of hormone-depletion operations and other forms of therapy for prostatic and mammary cancer, and survival times following various types of treatment for prostatic cancer.

In view of the "corticoid-like" properties of glycyrrhizin in the maintenance of adrenalectomized patients, it is presently being assayed for antiphlogistic properties and for its effect on the hypophysis.

New animal surgery facilities are being utilized at Delafield in an attempt to develop more satisfactory surgical techniques for surgical procedures on the bladder in cancer patients.

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Scientific Report of the Institute of Cancer Research

1956 - 1957

College of Physicians and Surgeons

Columbia University, New York

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Introduction

This, the sixth annual Scientific Report of the Institute of Cancer Research is dedicated to fellow workers in other institutions and to the individual donors, foundations and Governmental agencies which have helped to support through grants the work summarized in the succeeding pages. The reports here included have been prepared by the investigators involved in the work and the evidence amassed during the year constitutes, we hope, an eloquent expression of appreciation for the financial aid provided by so many.

The growth of public support for cancer research during the past two decades has been tremendous and ever increasing. One of the consequences of sustained grants-in-aid is continuity of research effort on a particular problem. This is well demonstrated by the cancer research activities at the College of Physicians and Surgeons. Much of the present report is devoted to the description of findings in ongoing programs which have been discussed in previous years. This indicates that broad areas of investigation have been selected within which systematic study can be profitably conducted for a number of years. In addition to the continuity of research on a program, there is strength provided through the sustained efforts of investigators who can give their individual attention to investigations with the reasonable expectation that their financial support will be continuous. In both laboratory and clinical cancer research here, there is a strong core of investigators; in addition to their research these men and women provide training to others who come for periods of one to five years and then go on to initiate their own programs. The combination of research and training for research is, I believe, an excellent way to invest funds for investigation since it provides an immediate dividend in the form of increased knowledge gained from the programs under way and it

provides a long term security through the training of future contributors to knowledge of cancer and its control.

Alfred Gellhorn, M.D.

June, 1957

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Biochemistry

At the Francis Delafield Hospital, Dr. Samuel Graff and his associates are engaged in a variety of studies which are integrated toward the common problem of growth and development.

a) *In vitro* tissue or cell cultures provide opportunities for detailed studies of metabolic events in pure cell strains under conditions which are dissociated from host influences. These cells are immortal and propagate logarithmically under the ideal conditions which have been approximated in the continuous cytogenerator developed by Dr. K.S. McCarty. This device has now been in successful operation for some time, serving both as a stock repository for viable cells and for studies in intermediary metabolism under exquisitely controlled conditions. This apparatus, the technical equivalent of a heart, lung, kidney preparation, avoids the discontinuities and fluctuations of static flask culture and makes possible the propagation of masses of cells sufficient even for N^{15} studies. It is significant that ascites cells have been cultured *in vitro* (in logarithmic phase) for the first time by means of this device. Application to screening studies are obvious and the device is already in operation in several laboratories for that purpose.

Experiments with isotopic tracers, including both C^{14} and N^{15}, are yielding information on biochemical events in cultured cells and on the influence of extrinsic factors on the selection of alternate metabolic pathways.

b) Growth, which entails biosynthesis, depends on a series of coupled endergonic reactions. Dr. Aaron Freedman, utilizing isotopic tracers, is engaged in the study of the bioenergetic pathways in the intact animal, and how they are influenced or regulated by nutritional status, hormones, and other intrinsic or extrinsic factors. His

experiments involved detailed studies of tricarboxylic acid cycle turnover and activity. His findings support the conclusion that the tricarboxylic acid cycle is an important contributor of protoplasmic constituents as well as a medium of energy transfer. The significance of these findings to the specific problem of tumor growth is the subject of continued investigation.

c) The energy requirements for metazoan biosynthesis are mainly satisfied by the oxidation of dietary fats and carbohydrates by way of glycolysis, the tricarboxylic acid cycle and the electron carrier chain which terminates with molecular oxygen. Diminution, within certain limits, of oxygen is compensated for by physiological and anatomical adaptations which tend to increase efficiency of delivery of oxygen to tissues even in the face of decreased ambient oxygen tension, and by cellular adaptations which enable cells to at least carry on maintenance reactions even though less oxygen is delivered to the tissues. Dr. Max Eisenberg is investigating the biochemical features of cellular acclimatization to partial hypoxia. This phase of acclimatization appears to involve a selection in metabolic pathways including enhancement of glycolytic mechanisms in compensation for diminished carrier chain activity.

d) Biosynthetic activities are diminished when animals are forced to reside under decreased oxygen pressures. The imposition of a degree of hypoxia tolerable to the host by virtue of acclimatization brings about an inhibition of the rate of growth of tumors in animals. Our collaborative experiments with Drs. A. Barach, H. Bickerman, and G. Beck of the Department of Medicine, on tumor inhibition by tolerable hypoxia continue to be fruitful. The tumor-inhibitory effects of hypoxia therapy have been confirmed many times. The relation of rate of acclimatization, persistence or reversibility of the phenomenon, and the clinical criteria of

acclimatization are under exploration. Both the degree of hypoxia to which an animal may be acclimatized and also the response of tumors to hypoxia are species or type characteristics respectively. That hypoxia therapy may be profitably applied to the human is suggested by the experiences of mountain climbing expeditions; the last Everest expedition established base camp at an altitude of 21,000 feet, which was considered to be within the limits of human acclimatizability.

e) Chemotherapy continues to develop largely along empirical lines. What is sought are reagents which will selectively interfere with biological mechanisms which are either qualitatively characteristic of tumors or quantitatively preponderant in tumors. Such selectivity is often the result of subtle structural nuances. This laboratory, therefore, some time ago embarked on a systematic program of synthesis of heterocycles whereby different substituents in different allocations could be compared for biological activity. Dr. H. B. Gillespie and Mr. Francis Spano have extended their synthesis of new benzotriazoles, benzimidazoles, and quinoxalines with a report on the synthesis of 3,4-diamino-5 nitrophenetole and 3,4-diamino-2-nitroanisole, because nitro substitution often appears to endow such compounds with biological activity. About 60 new compounds have been prepared so far in this program. Many of them are under biological tests in several laboratories, both here and abroad in several different systems.

f) The developing Rana pipiens embryo is a useful test subject for study of biological inhibitors because it is comprised of a closed system which develops in observable stages permitting a comparison of cell cleavage without synthesis with endergonic post-gastrular differentiation and synthesis. Dr. Kathe Liedke has made further progress in her application of the substituted heterocyclic compounds listed above to developing R. pipiens embryos. The finding, cited in a previous report, that

5-methoxy-7-nitroquinoxaline and 4-methoxy-6-nitrobenzotriazole were stage specific in that the former compound interfered with cell cleavage primarily, whereas exposure to the latter compound preferentially inhibited older differentiation stages, has enabled her to pursue the problem of specificity in detail by means of tissue transplantation and explantation. The possibility of reversibility or release from inhibition, and the effects on future cellular events of transient exposure to the agents have been under scrutiny. Not only do these experiments sharply illustrate the influence of chemical structure on biological reactivity, they also demonstrate that there are qualitative differences in the enzymatically catalyzed reactions leading either to cell division or to differentiation and growth.

g) Following the demonstration of MacDowell that the incidence of leukemia in C58 mice can be decreased markedly by foster-nursing the young on old Storrs-Little mothers, this laboratory undertook to isolate the effective substance from the milk of old Storrs-Little mice. The program necessitated establishing three highly-inbred strains in our laboratory, the C58 strain in which leukemia is endemic and which serves as our test strain, the Storrs-Little strain of which old females supply the milk, and the Bagg Albino, a vigorous neutral strain without which we could not rear the Storrs-Little strain.

A large colony of the Storrs-Little strain is operated as a dairy from which we now obtain approximately 1500 ml of milk from females of 11 months of age or over per year. Suitable experimental preparations of this milk are administered to young C58 mice and the incidence of leukemia as it develops is determined by clinical signs and histological examination. Mrs. Ada Graff, who is responsible for the processing of the milk and for the observations, has demonstrated that the active substance is of low molecular weight, i.e., that it is ultrafilterable. It is heat-labile and efficacious

only when it is administered early in life. Titration of activity was not decisive, not only because the expression of leukemia is highly variable in time in untreated C58 mice, but also because the route of administration, usually parenteral, inactivation by metabolic processes or by isolation procedures, and the decrease of effectiveness with the age of the recipient are also imponderable variables. Experiments on its chromatographic properties are still in progress. Evidence has been adduced elsewhere that leukemia in the C58 strain has a viral etiology and so it can be hypothesized that the protective substance is either an anti-viral agent or that it acts by way of protecting the virus host by prevention of cellular malignant mutation.

h) Dr. Victor Ross is investigating possible qualitative or quantitative differences between the serum proteins of animals inoculated with Ehrlich ascites tumor cells and those of uninoculated animals in a search for host reactions to tumors which might be of diagnostic value. Electrophoretic studies of the blood sera of rats and mice after intraperitoneal inoculation of ascites cells by means of the moving boundary method are in progress. These studies utilize several different buffer systems in order to make certain that slight changes which might not be apparent in one buffer could be detected in another, and so that the relative concentrations of the several serum proteins, which apparently vary somewhat according to the buffer system used, could be evaluated with less error.

Data are now available for the blood sera of rats inoculated with mouse ascites cells beginning with the third day after inoculation and daily thereafter to the tenth day when young adult rats are generally fully recovered. Increasing amounts of ascites fluid and multiplication of cells are apparent from the third to the fifth or sixth day after inoculation.

α_2-Globulin increases greatly in the blood of the rats at the time of maximal tumor cell growth, and there is also an increase in two fast-moving

components with mobilities somewhat greater than that of albumin in the sera of inoculated rats and mice. Small amounts of these fast-moving components can be detected in normal rat and mouse sera when careful experimental techniques are employed. There is also a fall in albumin concentration following tumor transplantation in both species of animals. Electrophoretic examination of the ascites fluid and of tumor cell extracts have also been made. The data are as yet insufficient to identify any of the tumor cell proteins with blood serum components, but it appears, tentatively, that the electrophoretic mobilities of several of them are not dissimilar to those of some of the serum proteins of both mice and rats.

Also at the Francis Delafield Hospital, Dr. Philip Feigelson and Mrs. Muriel Feigelson have continued their studies on the cellular mechanisms controlling the enzyme levels within mammalian cells. The roles of the nucleic acids, RNA and DNA, were investigated in adult rats during the substrate-induced elevation of tryptophan peroxidase. It was found that intraperitoneal tryptophan administration caused a nine-fold elevation in the liver tryptophan peroxidase activity reaching its maximum levels at five hours following the amino acid injection; the enzyme level then rapidly fell reaching control levels at 15 - 20 hours post-injection. The incorporation of radioactive inorganic phosphorus into the RNA and DNA of these livers was studied during various phases of this enzyme protein induction. It was found that during 0 - 5 hours post-tryptophan injection little or no stimulation in RNA or DNA synthesis occurred, however at the 10 - 15 hour period a three-fold elevation of RNA turnover was repeatedly noted, the DNA remaining essentially uninfluenced. Thus, there seems to be a marked stimulation of RNA synthesis which follows the enzyme protein synthesis. Our current working hypothesis is that RNA is a reactant which is consumed during enzyme protein synthesis and that this consumed RNA is

replaced subsequent to this period of active protein synthesis. These studies are currently being extended investigating the turnovers of the RNAs of the various subcellular components during these processes.

Dr. Feigelson has continued his studies on the nature of the xanthine oxidase catalyzed oxidations and the mechanisms of enzyme inhibition by chemotherapeutic agents. This enzyme catalyzes the oxidation of both hypoxanthine and xanthine to uric acid. Kinetic spectrophotometric studies demonstrated the accumulation of free xanthine during the oxidation of hypoxanthine to uric acid, thus indicating the more rapid dissociation of the enzyme-xanthine complex than the conversion of the complex to uric acid and free enzyme. A kinetic expression was developed for the overall reaction sequence which indicates that under steady state conditions, such as would prevail *in vivo*, the xanthine concentration should be approximately 70 per cent that of the steady state hypoxanthine concentration. Thus free xanthine is generated and may be available to serve as an *in vivo* reactant in anabolic reactions.

In hope of gaining insight into the nature of the substrate binding sites as well as the catalytic center of xanthine oxidase, the Michaelis constant (K_M) and the rate of conversion of the xanthine-enzyme complex to uric acid and free enzyme (k_3), were studied as a function of pH. As the pH was raised from pH 6 to 10 a gradual four-fold increase in k_3 was observed. Over the pH range of 6 to 8.5 the K_M varies but slightly, however as the pH is raised from 8.5 to 9.7 there is a twenty-five-fold increase in K_M indicating a dramatic decrease in the affinity of the substrate for the catalytic center of the enzyme. This is the pH at which the free NH_3+ groups in proteins ionize losing their positive charge. As xanthine is negatively charged at these pH's it may be inferred that coulombic attractions between the negatively charged substrate and positively charged NH_3+ groups of the enzyme surface are primary in binding the

substrate to the enzyme; likewise since no such alterations in k_3 took place this indicates that the NH_3^+ groups of the enzyme protein are not directly concerned with the catalytic activity itself but rather implies that they are concerned exclusively with binding the substrate to the enzyme prior to its catalytic transformation.

Enzyme inhibition studies employing certain pyrazolo(3,4-d)pyrimidines demonstrated that these compounds are competitive inhibitors of both purine and aldehyde oxidations by xanthine oxidase, thus indicating that both classes of compounds are oxidized at the same active center of the enzyme.

In the laboratories at the College of Physicians and Surgeons, Dr. Erich Hirschberg has continued his participation in the interrelated program of research on the pathophysiology and chemotherapy of neoplastic disease described in a later section of this report. In addition, he is pursuing with Miss Eva Landesman experiments concerned with differentiation in the slime mold Dictyostelium discoideum. The first phase of differentiation in this organism is the aggregation of the previously independent amoebae to form a multicellular pseudoplasmodium. This process is initiated and controlled by an unidentified chemotactic principle, called acrasin, produced by the central amoebae. Acrasin is present in very low concentrations and is unstable under normal conditions. Much of the work of the past year has been devoted to attempts to elucidate the chemical nature of this chemotactic principle.

Freshly collected fluids containing acrasin give positive reactions in spot tests with ninhydrin, biuret, and the Pauly reagent for imidazoles. Hydrolyzates of acrasin concentrates yield spots corresponding to several amino acids in paper chromatograms. Acrasin concentrates exhibit an ultraviolet absorption spectrum with a peak in the region of 255-260 mμ, while concentrates of analogous fluids collected from cultures of the slime

mold prior to the appearance of acrasin do not give the same absorption spectrum. A number of methodological problems, particularly the development of a reliable and rapid biological test for acrasin, are currently under investigation.

Dr. Stephen Zamenhof has studied the effects of introduction of 5-bromouracil into DNA of E. coli on the heredity of the cell. In certain cases it was found that this produces high mutation rates and can unstabilize the genes. Spontaneous gene labilization and stabilization was studied for comparison. In some cases the unstable genes are not eliminated by natural selection and may play an important role in the evolution.

In another project, Dr. Zamenhof and Dr. Sheldon Greer have found that the cells containing 5-bromouracil in their DNA are up to 3000 times more sensitive to ultraviolet irradiation. These studies were carried out principally with thymine-requiring mutants of E. coli. He has investigated the conditions for introduction of 5-bromouracil into DNA of wild strains of E. coli.

Dentistry

Studies currently under investigation in the Department of Dentistry of Francis Delafield Hospital continue to be concerned primarily with the clinical and laboratory evaluation of various pharmacotherapeutic agents as well as a group of problems in the fields of oral pathology, physiology, and bacteriology. A number of the studies described in the previous year's report have been completed and appear as literature references in this year's bibliography. These studies and those described now in progress have been undertaken by Drs. John D. Piro, Edward V. Zegarelli, and Austin H. Kutscher.

Current investigations include: studies in humans concerned with the subcutaneous pain threshold with pressure using the Hardy-Wolff-Goodell algesimeter; histopathological study and picture atlas of patients with "familial white folded hypertrophy of the oral mucous membranes", with the Department of Pathology; continuation of a previously published disc sensitivity study including newer and also previously available antibiotics (including thiostrepton, albamycin) in terms of all organisms isolated in the bacteriological laboratory, with the Department of Bacteriology; continued evaluation of a reported relationship between the occurrence of torus palatinus and breast or uterine carcinoma; an evaluation of various denture adhesives; studies directed at demonstrating physiological and mechanical activity of India ink; continuation of previously published studies evaluating a series of new local anesthetic agents (Carbocaine and Squibb MC 2252) in terms of toxicity findings following their intraoral injection into mucobuccal fold areas - in association with the Department of Pathology; similar study of topical anesthetics (Vesprin, Ophthaine, Squibb MC 2653, Carbocaine and antihisteminic agents) following topical application to the mucobuccal fold area; evaluation of mycostatin

and fluorohydrocortisone in the treatment of denture sore mouth, cheilosis, and glossitis in the normal human, hospital personnel, debilitated and terminal carcinoma patients; studies in humans of the efficacy, allergenicity (patch and scratch tests) and histopathology of cultured calf bone following implantation into sockets, with the Department of Pathology; evaluation of the physiological and pharmacological effects of epinephrine comedication in causing reactions when injected with local anesthetic agents during dental clinic and operating room procedures, with the Department of Anesthesiology; effectiveness and usefulness of a new local anesthetic agent, Carbocaine, including study of toxicity, the effect on vital functions, circulating blood titers, urinary excretion, intranasal allergy testing, with the Department of Anesthesiology; effectiveness and systemic pharmacological activity of a new topical corticosteroid, 9, 21-difluorodesoxyhydrocortisone; efficacy and local side reactions of a new non-absorbable (through the gut) antibiotic (thiostrepton) with a penicillin-type spectrum in terms of oral infections; attempts to pass local anesthetics through the oral mucous membranes by means of iontophoresis; usefulness and evidence of tissue reaction following the application of a new spray plastic dressing; attempts at inactivation of topical anesthetics by means of various pharmacotherapeutic agents including cholinesterase, spreading agents, vasodilators, and directed subsequently at parallel studies on injectible local anesthetics and attempted subperiosteal implantation of dental crown and bridge prostheses.

The following studies have been completed: Studies of some problems encountered during mass dental extractions on the adrenalectomized patient; continuation of disc sensitivity studies following upon previously published data including an evaluation of the efficacy of combinations of antibiotics; histopathology of areas treated with carbocaine and lidocaine; percentage and incidence of negative reports appearing in the medical literature - a

review of the literature; a picture atlas of Peutz-Jegher's syndrome (oral pigmentation with associated intestinal polyps); histopathology of lesions in patients with recurrent ulcerative stomatitis and recurrent scarifying ulcerative stomatitis.

Gynecology

Work on gynecological cancer is carried on in the wards and clinics of the Presbyterian and Francis Delafield Hospitals and in the laboratories of the Francis Delafield Hospital and the College of Physicians and Surgeons. The clinical care of patients, although shared by all members of the Department of Obstetrics and Gynecology, has been the particular responsibility of Dr. Saul Gusberg, Albert Plentl, Equinn Munnell, Henry Clay Frick, David Moore, Harold Tovell, Robert Hall, Mary Jane Gray, and Howard Taylor. The laboratories have been under the direction of Dr. Earl T. Engle, Dr. Seymour Lieberman, Dr. Joseph Jailer and Dr. Saul Gusberg.

The report on the types of work being conducted may properly be divided under the following headings:

1. The relationship of the steroid hormones to cancer.
2. The biology of the differentiation of ovarian and endometrial tumors.
3. Histological and cytological measures of radiosensitivity.
4. Early diagnosis and precancerous lesions.
5. The investigation of clinical therapeutic methods.

1. The relationship of the steroid hormones to cancer

The work in the laboratory of Dr. Seymour Lieberman has been characterized during the year by attention to basic principles rather than to immediate applications to the cancer problem. Work has been continued on the nature of the urinary 17-ketosteroids excreted by normal individuals and by patients with a variety of diseases, including cancer. The excretion of 17-ketosteroids by 15 normal men and 10 normal women was determined in order to establish the normal limits usually found in the urine. The daily individual variations in the excretion of 17-ketosteroids by some of these subjects was also measured.

is increased sensitivity to exogenous ACTH. This has been demonstrated in 14 of 15 patients with this condition. On the other hand, in five of six patients whose Cushing's syndrome was due to an adrenal tumor, no such exaggerated response to ACTH was found and no "adreno-corticotrophic" activity could be demonstrated in the plasma.

Another technique which can demonstrate relationships between hormones and cancer depends on morphological studies of the ovary and the endometrium. Adenomatous hyperplasia of the endometrium occurs in women in the child-bearing age and is associated with menstrual abnormalities and infertility. This group of patients as well as a group with milder types of hyperplasia associated with menstrual abnormalities are being intensively studied by Dr. Anna Southam. Various types of progestational drugs are being used in an attempt to control systems and modify endometrial patterns, but have been successful only in short term control of symptoms. Reversal of the hyperplastic endometrial picture to normal has been obtained with partial resection of the polycystic ovaries associated with this lesion.

The systematic study of the ovaries by means of serial sections and a formula by which the total amount of functioning granulosa and theca tissues may be calculated has been continued. The material now collected consists of several thousands of measured tissue sections. Preliminary survey of the collected data indicates the possibility that the ovaries of young women with cancer of the cervix may exhibit an unusual amount of functional activity. The final analysis of this material is now under way and it is hoped will be finished within the coming year.

2. Studies of the biochemistry and biology of tumor differentiation

The study of the biology and biochemistry of the grades of malignancy of cancer of the endometrium and of the ovary is progressing. The program

comprises the work of Margaret Long, Ph.D., Helena deRoetth, Ph.D., Saul Bader, Ph.D., and Hyman Guthwin, Ph.D. Dr. Harold Tovell is carrying on some work in association with this program and its general organization is under the direction of Dr. Howard Taylor and Dr. Seymour Lieberman. Acknowledgement is also made for the advice and collaboration of Dr. M. J. Kopac in the Department of Biology at New York University.

Dr. Long's particular work has been the survey of the histochemical characteristics of different grades of malignancy. A total of 279 gynecological tumors have been examined during the last four years. These include 82 papillary serous and 39 pseudomucinous ovarian carcinomas, 50 endometrial carcinomas, 55 cervical epidermoid carcinomas and 53 miscellaneous gynecologic neoplasms.

The work reported a year ago on the nucleolus in ovarian carcinoma has been extended to the endometrium, with the analysis of 77 cases of non-malignant endometrium and 40 cases of endometrial carcinoma. This work has been carried on under Dr. Long's direction by Dr. Fumio Doko. It has again been found that with increased grades of malignancy the size of the single nucleolus increases and that there are larger numbers of multiple nucleoli and of nucleoli with irregular forms. These observations suggest an increase in nucleolar ribonucleic acid as loss of differentiation progresses.

A second cytochemical study has been completed this year. This concerns the localization of the enzyme succinic dehydrogenase in ovarian papillary serous tumors. The site of the enzyme, as demonstrated by types of formazan deposits, was, however, found to be the same in the six cases of benign ovarian tumors as in the 30 cases of cystadenocarcinoma of different degrees of differentiation. In contrast to the work of ribonucleic acid, therefore, the current studies of succinic dehydrogenase activity have produced no evidence of correlation with grades of malignancy.

Dr. Helena deRoetth has extended her studies of the metabolic behavior of human gynecological tumors in vitro. In the human ovarian papillary serous tumors, a correlation has been found between the histologically determined grade of malignancy and the amount of oxygen consumed and lactic acid produced. A similar correlation was found for the oxygen consumption of human pseudomucinous cystadenocarcinomas, but no such correlation was evident in tumors of the cervix. Dr. deRoetth's results indicate that, in a general way, the more malignant the papillary serous tumors the more glycolysis occurs.

During all of the previous work on differentiation of tumor tissues, the great problem has been the miscellaneous character of the material. Carcinomas as obtained at the operating table include, beside specific viable malignant cells, connective tissue and blood vessels as well as areas in which the tumor tissue may be more or less degenerated. Such heterogeneity makes calculations very difficult. Accordingly, Dr. Hyman Guthwin has begun a long-range program designed to fractionate human gynecological tumors into various cellular components. He is attempting first by differential centrifugation to isolate pure samples of tumor cells which then may be studied by a variety of chemical and cytological methods. Further work will attempt the division of the cells themselves into fractions containing specific subcellular particulates.

Working with samples from the same human tumor material, Dr. Saul Bader is carrying out quantitative measurements of the nucleoproteins of the cells by means of absorption microspectophotometry. Dr. Harold Tovell with a smaller number of tumors is studying the problem of heterologous transplantation.

3. The radiosensitivity of cancer of the cervix

In attempting to recognize degrees of radiosensitivity of cervical

cancer Dr. Saul Gusberg and his group believe that during the past five years they have acquired sufficient data to enable them to analyze their results in a preliminary way. They are now able to predict radiosensitivity with reasonable accuracy by means of serial tumor biopsies taken after a test dose of radiation. Studies by these methods are being compared with the results of the purely cytological test developed in Boston by Drs. Ruth and John Graham.

Analysis of the data collected up to this point suggests the possibility of an endocrine basis for some of the cytological observations, but the follow-up on these patients is still too short to permit a final evaluation of this test as a method of predicting radio curability. An associated investigation in Dr. Gusberg's laboratory is being carried out by Dr. Helena F. Hughes who is studying the vaginal smears of patients who do not have gynecological carcinoma, to determine the relationship between certain morphological changes in basal cells, reported elsewhere as significant in radiosensitivity testing, and simple factors of hormonal status.

4. The early diagnosis and the study of precancerous lesions

Dr. Gusberg and his group have also continued their interest in the precursors of uterine cancer with special emphasis on adenomatous hyperplasia of the endometrium and also epithelial cancer of the cervix. With Dr. Southam, Dr. Gusberg is studying the relationship between a certain type of cystic ovary ("The Stein-Levanthal ovary") and the development of hyperplasia and cancer of the endometrium.

Reference should also be made to the continued effort to evaluate the Papanicolaou vaginal smear test as a diagnostic and screening method for the detection of uterine cancer. During the last year the cytology laboratory of the gynecology division at the Presbyterian Hospital carried out the

examination of 6,875 vaginal smears with a detection of 29 cases of intra-epithelial cancer of the cervix. The frequency of positive smears, about 0.4 percent, does not refer exclusively to symptomless cases.

5. Clinical investigation of therapy of gynecologic cancer

The techniques for the treatment of cancer of the cervix on the gynecological services of the Presbyterian and the Francis Delafield Hospitals were outlined in a relatively standardized form by a series of conferences between the Departments of Radiology and Gynecology held in the fall of the year 1951. The first program was put into effect on January 1, 1952. A similar series of conferences was held in the fall of 1956 with the purpose of considering in what way the experience of the first five years' work under the program should modify the program for the future. As a result a revised program was prepared, mimeographed and put into effect on January 1, 1957.

The purpose of the program has been to make possible a collaborative study to include the radiological and gynecological divisions of both the Presbyterian and Francis Delafield Hospitals. In working up the program several points were taken into consideration. First it was realized that a degree of consistency must be maintained for a long enough period of time to permit decisions to be made as to the relative efficacy of different procedures. Secondly, it was believed that where alternative methods of treatment existed, with the same claims as regards cure rates, both methods should be tried for purposes of comparison.

The Columbia Presbyterian Medical Center is admirably organized for the study of therapeutic methods. Radiological and surgical techniques can each be applied by experienced hands. The varieties of external x-ray therapy available, namely the cobalt-60 apparatus, the 2000 KV x-ray machine

and the betatron offer special opportunities for making contrasts.

The amount of gynecological cancer work at the Medical Center apparently increases continually. Of interest in this respect is the following table showing the number of cases of various types of gynecological cancer treated at the Presbyterian Hospital in 1946 for comparison with the list for 1956 at the Presbyterian Hospital and a second list for 1956 at the Francis Delafield Hospital. The annual case load of over 300 cases is a measure of the opportunities being offered for the advancement of therapeutic knowledge in this field.

Annual New Cases of Gynecological Cancer

	Presbyterian Hospital		Francis Delafield Hospital
	1946	1956	1956
Cancer of cervix - intra-epithelial	0	29	6
Cancer of cervix - invasive	37	50	93
Cancer of body of uterus	38	34	22
Cancer of ovary	16	24	35
Others	6	21	16
	97	158	172

The investigation of therapeutic methods in gynecologic cancer continues with its principal emphasis on the relative merits of radical surgery and radiation therapy in stages I and II of cancer of the cervix and the need of preoperative radiation of cancer of the endometrium. As a result of the experience gained in the first five years, however, it has been at least tentatively decided to discontinue any radical surgery ("exenteration operations") on stage III or stage IV cases of cancer of the cervix. As

a further result it has become apparent that the surgical removal of the regional lymphatics after radium and x-ray therapy for primary carcinoma of the cervix has produced more operative complications than is justified by the theoretical possibilities of an improved cure rate.

Mention should finally be made of the improvement in the organization for the clinical follow-up of treated cases of gynecological cancer. The determination of the final results of treatment is obviously essential to any scientific investigation of therapeutic measures and particular credit should go this year to Dr. David Moore who has worked very hard in perfecting our follow-up system.

Medicine

In last year's report, the interrelated efforts of this group to develop a broad program of research on the pathophysiology and chemotherapy of neoplastic disease were discussed in some detail. Many of these studies are continuing and several new projects have been initiated. The principal areas as well as the professional personnnel involved in their exploration are:

Tumor Screening and Pharmacology

A. Gellhorn, M. D.

E. Hirschberg, Ph. D.

Mechanism of Drug Action

J. D. Davidson, M. D.

P. Feigelson, Ph. D.

Metabolism

P. Marks, M. D.

J. Bishop, M. D.

Hematology

C. Hyman, M. D.

J. Ultmann, M. D.

Myeloma

E. Osserman, M. D.

Cardio-pulmonary Physiology

H. Heinemann, M. D.

C. Emirgil, M. D.

Clinical Chemotherapy

A. Gellhorn, M. D.

W. Bahnson, M. D.

D. Choy, M. D.

C. Crandall, M. D.

A. Edwards, M. D.

C. Emirgil, M. D.

W. Fish, M. D.

G. Hyman, M. D.

J. Mijnssen, M. D.

H. Ranney, M. D.

T. Richter, M. D.

S. Suh, M. D.

H. Thompson, M. D.

J. Ultmann, M. D.

Tumor screening and pharmacology

a) Experimental tumor testing - The transplantable mouse or rat tumor is still the most extensively used test object in the discovery and

evaluation of new agents of potential clinical interest. In this laboratory, Drs. Alfred Gellhorn and Erich Hirschberg together with Mrs. Shirley Brindle and Misses Janet Hibbert, Jane Merselis, and Gloria Semente have continued to test each new compound against a wide spectrum of transplanted mouse tumors, including principally two mammary adenocarcinomas (755 and Eo771), an undifferentiated tumor (S180), a brain tumor (Glioma 26), and the Ehrlich ascites carcinoma. For the solid tumors, the final weight of tumors removed from groups of twenty mice treated with the agent by daily intraperitoneal injections at the maximum tolerated dose levels is compared with the tumor weight of control animals; for the ascites tumor, increase in survival time after a total of three injections of the compound under investigation is used as the sole index of tumor inhibition.

An evaluation of a small series of representative acridines related to the antimalarial quinacrine has been completed; these compounds did not exhibit significant carcinostatic activity *in vivo*. About three dozen quinolines, including the antimalarials chloroquine and camoquin, have been tested, with particular attention to compounds differing from camoquin only by slight alterations in side chain or ring substituents. Some anticancer activity has been observed with camoquin and several close congeners, but the degree and consistency of inhibition has not been impressive. Chloroquine is inactive against the tumors included in this spectrum.

Particular emphasis has been placed in the last year on a group of nitrogen mustard derivatives and other potential alkylating agents. The compounds under investigation have included several aromatic and heterocyclic derivatives developed and studied in other laboratories, for example, quinacrine, camoquin, and chloroquine mustards and p-di-(2-chloroethyl)aminophenylpyruvic acid (chlorambucil), and a series of new nitrogen mustards synthesized

for this program by Dr. W. S. Gump of the Givaudan Corporation. The compound of greatest interest has been the benzimidazole mustard, 2-[di-(chloroethyl)-aminomethyl] benzimidazole, in which the reactive bifunctional alkylating moiety is attached to a potential purine antagonist. This new agent inhibits not only the Ehrlich ascites carcinoma, which responds readily to many alkylating agents, but also the two mammary carcinomas and Sarcoma 180, in contradistinction to the "antimalarial mustards" and other alkylating agents included in comparative experiments.

b) Preclinical pharmacology - In view of the activity shown by benzimidazole mustard in the experimental mouse tumor spectrum and of its theoretical interest as a first attempt to use a purine antagonist as the carrier for the alkylating function, the toxicology and side-effects of benzimidazole mustard in mice, rabbits, and dogs have been examined with some care. In mice, higher doses given for five consecutive days caused progressive weight loss and some delayed deaths. Qualitatively, the toxicity resembled that of other nitrogen mustards and not that of benzimidazole. Histological examination of liver, kidney, and sternum of tumor-bearing mice after administration of lower, tumor-inhibitory doses failed to yield any evidence of toxicity to these host tissues. Essentially the same conclusions were drawn from experiments with fourteen rabbits and eight dogs. The permissible dosage limits in man for both intravenous and oral administration of benzimidazole mustard are being determined by Drs. Alfred Gellhorn and John Ultmann.

c) Experimental chemotherapy of brain tumors - The interdisciplinary investigation of approaches to chemotherapy of the malignant brain tumor, glioblastoma multiforme, in man continues to engage the attention of Drs. Gellhorn and Hirschberg and Mrs. Martha Osnos in collaboration with Dr. Margaret R. Murray and Mrs. Ruth Eising of the Laboratory of Cell Physiology and Drs. Hartwell Thompson, Joseph Ransohoff, and J. Lawrence Pool of the

Neurological Institute. This investigation has comprised three general areas in addition to the *in vivo* screening of compounds against mouse glioma 26 mentioned above: tissue culture experiments with this and other mouse brain tumors and human brain tumors and embryonic tissues; clinical evaluation of promising agents; and analytical studies of drug distribution in the tissues of both mice and patients. A new area of research, which has been added during the past year, deals with diagnostic problems in this type of neoplasm.

In vitro experiments with all compounds which have also been studied *in vivo* indicated that, in general, the tumor cells in tissue culture respond more readily to acridines, quinolines, and related compounds than to nitrogen mustards and other alkylating agents. Structure-activity relationships in the quinoline series are not always in agreement with data obtained in the mouse tumor spectrum; for example, camoquin and chloroquine exhibit the same order of tumor-inhibitory activity *in vitro* whereas only the former has any effect *in vivo*.

Camoquin (amodiaquin) has now been evaluated clinically in a small series of patients with glioblastoma multiforme, with generally discouraging results. This quinoline antimalarial, then, is no more active against this type of neoplastic growth in man than the acridine antimalarial quinacrine.

It was shown last year that the tissue levels of quinacrine which could be built up in the brain tumors of patients by long-term oral administration of maximum tolerated doses of quinacrine fell short of the concentrations found in tissue culture explants after exposure to minimum effective doses of the drug. In an attempt to increase the quinacrine concentrations in these tumors *in vivo*, the intracarotid arterial route of administration is currently being explored. In a terminal patient exposed to quinacrine for only a few days, the drug concentration in the tumor at autopsy was slightly higher than the highest level previously achieved by oral administration,

while the concentration in other tissues was the same or lower than in the earlier experiments.

One of the particularly pressing problems in the area of human brain tumors is the development of further tools for early and definitive diagnosis. In the last few months, a Grass electroencephalograph has been acquired; Dr. Thompson is currently preparing to add this new parameter to the study of the natural history of the disease and to make available another criterion in the objective evaluation of its treatment by chemotherapy. In another facet of this study, the usefulness of the determination of serum and spinal fluid phosphohexose isomerase levels as a guide to the progression of the human glioblastomas is being evaluated by Drs. Gellhorn, Hirschberg and Thompson. Available evidence suggests that the activity of this enzyme is elevated in the spinal fluid in the presence of brain tumors.

d) Host physiology - One of the intriguing problems in cancer research which has not yet been studied exhaustively concerns the mechanisms by which the progressively growing tumor in man or experimental animals causes the debilitating changes in the metabolic performance of the host which eventually develop into terminal cachexia. Drs. Gellhorn and Hirschberg and Mrs. Osnos have initiated a series of experiments to explore this question from several points of view. The focal point of the study to date has been the liver of the host.

One of the processes which is of crucial importance to the cell is the complex of enzymatic reactions, oxidative phosphorylation. In view of the apparent similarity between the symptoms of dinitrophenol poisoning, which involves uncoupling of phosphorylation from oxidation, and those of terminal cachexia observed in certain neoplastic diseases such as Hodgkin's disease, an attempt was made to demonstrate an impairment of oxidative phosphorylation

in liver "cyclophorase" (R_3L) preparations or mitochondria from mice bearing progressively growing tumors. Some of the data indicated a slight drop in the P/O ratio of liver preparations once the tumor (carcinoma 755) had grown to a weight of at least a gram (10-14 days after transplantation), but the results were not consistent and the decrease was not sufficiently large even in the "positive" experiments to account for the terminal symptoms of the disease. An analogous series of experiments, using overall anaerobic glycolysis (lactic acid production) and some of the individual glycolytic enzyme reactions, is in progress.

In another approach to the problem, the ability of the liver of normal subjects and of cancer patients to acetylate a sulfa drug, 2-sulfanilamido-4,6-dimethylpyrimidine (sulfamethazine), has been assessed by determining the free and acetylated sulfonamide excreted in the urine following a single loading dose of the drug. Patients with extensive liver metastases and in a terminal state acetylate the sulfonamide normally.

Mechanism of drug action

Dr. Jack D. Davidson and Dr. Philip Feigelson have continued studies of the mechanisms of anti-tumor action, and resistance to this action, from the standpoint of nucleic acid metabolism.

Last year's findings of inhibition of the enzymes adenosine deaminase and xanthine oxidase *in vitro* by 8-azaguanine were studied for their *in vivo* significance by attempting to reverse the antitumor activity of the compound with purines. Mice bearing adenocarcinoma 755 under therapy with 0.33 mM/kg. 8-azaguanine were given guanine, hypoxanthine or xanthine in doses of 2 mM/kg. daily. The only suggestion of drug reversal in two complete studies was a very slight one by hypoxanthine in only one experiment. It is felt that these experiments failed to support the hypothesis that 8-azaguanine acts by

inhibiting xanthine oxidase.

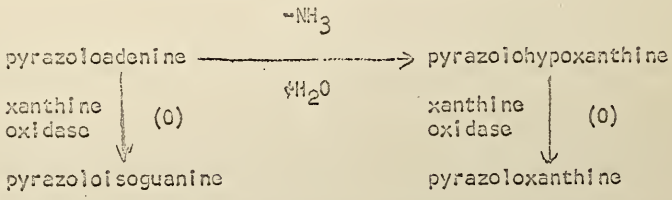
The biochemical mechanisms underlying the carcinostatic action of a new series of purine analogues, the pyrazolo(3,4-d)pyrimidines, were investigated by studying their effectiveness as *in vitro* inhibitors of certain isolated enzyme systems. It was found that various members of this series exerted strong *in vitro* inhibition of xanthine oxidase, with pyrazoloisoguanine exerting 50% inhibition at one millionth molar concentrations. Studies with these and other analogues indicate that the presence of functional groups at positions analogous to those substituted in the normal purine substrates of this enzyme, results in increased affinity for the enzyme active surface. Kinetic studies indicated that pyrazoloisoguanine competes with the normal substrate, xanthine, for the active center of the enzyme.

It was observed that pyrazoloadenine served both as a weak inhibitor of xanthine oxidation by xanthine oxidase and simultaneously as a substrate for the same enzyme. Apparently the pyrazoloadenine competed with xanthine for the enzyme's catalytic center and then underwent a rather slow enzyme-catalyzed oxidation to pyrazoloisoguanine. This is an interesting instance of an enzyme converting a weak inhibitor, pyrazoloadenine, to a very potent inhibitor, pyrazoloisoguanine.

Since published reports indicated the effective *in vivo* carcinostatic activity of pyrazoloadenine on certain experimental tumors it was deemed worthy to investigate the *in vivo* catabolism of this compound. Particular attention was paid towards ascertaining whether the xanthine oxidase catalyzed oxidation of pyrazoloadenine to pyrazoloisoguanine, which was demonstrated *in vitro*, also took place *in vivo*.

Following intraperitoneal administration of pyrazoloadenine to mature rats, their urines were collected and the excreted pyrazolopyrimidines were

isolated and subjected to paper chromatographic identification. The following catabolites were found: pyrazoloisoguanine, pyrazolohypoxanthine, pyrazoloxantnine and an as yet unidentified compound. To determine whether the biosynthesized pyrazoloxanthine arose via the oxidation of pyrazolohypoxanthine or via the oxidative deamination of pyrazoloisoguanine, experiments were undertaken in which pyrazoloisoguenine or pyrazolohypoxanthine were administered to rats. It was noted that rats given the latter compound did and those given the former compound did not excrete pyrazoloxanthine. Pyrazolohypoxanthine was therefore shown to be the precursor of pyrazoloxanthine. The enzyme responsible for the oxidative deamination of pyrazoloadenine remains as yet uncharacterized. In summary the rat metabolizes pyrazoloadenine as follows:



A considerable number of tracer experiments were done using L-1210 ascites leukemia cells *in vitro* to study the influence of 8-azaguanine and 6-mercaptopurine on the incorporation of P^{32}, guanine-C^{14} and hypoxanthine-C^{14} into the nucleic acids of these cells. The studies employed three strains of L-1210 which were respectively sensitive to, resistant to, and dependent upon the anti-purine drugs with respect to growth. In this system the drugs were found to have a very small inhibitory effect upon the incorporation of P^{32} even in the sensitive strain of tumor cells. This is in contrast to the marked inhibition seen in other tumors *in vivo* and may indicate either that the drugs have to be metabolized by other host tissues to yield active forms of the drug or that their inhibitory effects are masked in these two-hour

in vitro studies due to persistence of sufficient quantities of those intermediates whose synthesis is inhibited.

In the experiments with carbon-labeled purines as tracers the studies are still incomplete. It is apparent that 8-azaguanine does not interfere with the utilization of adenine to form nucleic acid adenine or guanine in any of three strains of L-1210. In the sensitive strain there is moderate interference with the utilization of guanine to form the adenine constituent of both RNA and DNA, though the latter conversion takes place to an insignificant extent even in the absence of 8-azaguanine. Guanine remains to be studied in the resistant and dependent strains. Very recently experiments have been done with hypoxanthine-C^{14}. Here again 8-azaguanine caused no interference but the extent of utilization was so unexpectedly great that studies were also made with 6-mercaptopurine since it is such a *bona fide* chemical analog of hypoxanthine. Preliminary data indicate that 6-mercaptopurine very greatly inhibits utilization of hypoxanthine for both adenine and guanine of both RNA and DNA, and that this occurs simultaneously with absence of inhibition of P^{32} incorporation.

The resistance of certain strains of tumors to purine analog compounds has often been attributed to impermeability of the resistant cells to the drug. This has been tested directly with the L-1210 ascites leukemia cells. Suspensions of sensitive cells and of 8-azaguanine resistant cells (cross-resistant to 6-mercaptopurine) were mixed *in vitro* with accurately measured amounts of (1) tritium water, (2) polyvinyl pyrrolidone stained with T-1824 dye and (3) either 8-azaguanine-2-C^{14} or 6-mercaptopurine-S^{35}. After equilibration of such mixtures for 10 minutes at 37°C. the tubes were centrifuged gently and the concentrations of all components in the supernatant solution were determined and compared with those in a blank tube containing everything but cells.

The distribution spaces for the drugs (3) were then compared with the total water space (1) and the extracellular space (2). Azaguanine was found to distribute to total water in the case of both the sensitive and the resistant L-1210 cells. With 6-mercaptopurine the apparent distribution space was found to be even larger than the total water after ten minutes incubation. This is interpreted to indicate metabolism, concentration, or binding of the compound and occurred with both strains of cells. When the experiment was repeated at 0°C., the drug space matched the tritium water space in the resistant cell system but still showed an apparent 20% greater dilution in the sensitive cell tube.

These incomplete studies offer strong evidence that 8-azaguanine and 6-mercaptopurine penetrate into cells of L-1210 leukemia that are resistant to the growth inhibitory action of the drugs.

Metabolism

a) Glucose metabolism in tumor-bearing hosts - Drs. Paul A. Marks and Jonathan Bishop have shown that a decrease in glucose tolerance, as indicated by a slower rate of disappearance of blood glucose following intravenous glucose administration, may be associated with the presence of malignant disease. This diminished carbohydrate tolerance was observed in patients with lymphoma, chronic leukemia and clinically early cervical and breast carcinoma. These subjects were carefully selected to exclude various other factors known to impair carbohydrate metabolism. No significant difference in the fasting blood sugar level or in the estimated volume of distribution of glucose was found between control individuals and the group of patients with neoplastic disease.

The diminished carbohydrate tolerance in the individual with malignant tumor might result from an alteration in the rate of release of glucose from

the liver, from a decrease in the rate of peripheral utilization of glucose, or from a disturbance in both of these metabolic processes. In view of the fact that the decreased glucose tolerance was associated with localized carcinoma, small in size relative to total body mass, it appears likely that the defect in carbohydrate utilization reflects alterations in host tissue metabolism associated with the presence of the neoplastic process.

During the past year, these studies have been extended in an effort to (1) evaluate the extent to which altered responsiveness to insulin may be associated with this impaired glucose tolerance in human subjects (2) determine the progress of this defect in glucose metabolism during the course of the malignant disease and, in particular, in relation to therapy and (3) study the alteration in host tissue carbohydrate metabolism in the rat bearing the Walker carcinosarcoma 256.

(1) The response to insulin has been evaluated in a group of 27 carefully selected patients with lymphoma, chronic leukemia, and carcinoma of the cervix and of the breast and in 12 control individuals.

A procedure was developed for the performance of the intravenous glucose-insulin tolerance test which permitted evaluation of a given individual's response to both the standard glucose load and a standard insulin dose in the same morning. This was done by administering,75 minutes after the intravenous dose of 25 gms. of glucose, glucagon-free insulin, 0.1 unit/kg I.V. From the curve of glucose disappearance from the blood a value K was calculated which represented the percent glucose disappearing per minute and was taken as an index of glucose tolerance during the period following glucose administration and of insulin tolerance during the period following insulin administration. This present procedure was found to yield reproducible results in normals and in patients with neoplastic disease.

It was found that the patients with malignant disease on the average

have a significantly lower rate of blood glucose disappearance following insulin administration than the control group (4.49%/min. compared with 6.80%/min. respectively). In this group of patients the average glucose disappearance rate was significantly lower than that of the control group, 1.14%/min. compared with 1.80%/min. respectively. These values are in agreement with the findings in our previous study of a larger group of individuals. These present studies indicate that in patients with neoplastic disease, in addition to an overall reduction in the rate of disappearance of glucose from the blood following an intravenous glucose load there is a reduced response to insulin. Recent investigation in the laboratories of several workers suggests that the early effect of insulin is that of increasing peripheral uptake and metabolism of glucose. In the present experiment the decreased rate of blood glucose disappearance following insulin administration is based on an analysis of the early portion of the curve of disappearance and suggests that the impaired carbohydrate tolerance associated with insulin administration in patients with malignant disease, in part at least, reflects decreased peripheral glucose utilization.

(2) The demonstration of a defect in carbohydrate metabolism associated with the presence of neoplasia led to studies of changes of this metabolic disturbance during the course of malignant disease and in particular in relation to therapy. Serial observations have been performed in 19 individuals with lymphoma or chronic leukemia and 7 individuals with initially localized carcinoma of the cervix and breast. In addition to the selection of patients according to the previously indicated criteria, follow-up studies were performed only in individuals in whom these criteria could be maintained.

Among the 19 individuals with lymphoma, 14 had objective though temporary remission associated with chemotherapy or radiotherapy. In none of these individuals, with one exception, a patient with chronic myelogenous

leukemia, was a remission associated with an improvement in glucose tolerance. The studies in four individuals with cervical carcinoma and in three individuals with breast carcinoma have revealed that in two of these patients the glucose tolerance has returned to normal. These individuals have been followed for only 12 and 18 months respectively and there is as yet no evidence of recurrent disease. In the remaining 5 individuals there has been evidence of recurrent disease and, in these patients, the abnormal glucose tolerance has persisted.

These follow-up studies are very limited and continued experiments are in progress both in this institution and in association with the Cancer Control Hospital in San Juan, Puerto Rico in an effort to increase the number of patients being followed with regard to the alterations in carbohydrate tolerance associated with effective therapy of malignant disease.

(3) In an effort to elucidate the mechanism of impaired carbohydrate tolerance of tumor-bearing hosts it would be desirable to be able to study this phenomenon under the more carefully controlled conditions possible in the experimental animal. Toward this end studies have been in progress to ascertain whether an alteration in carbohydrate tolerance similar to that observed in human subjects is associated with the presence of the Walker carcinosarcoma 256 in the rat.

Rats which have been carefully selected to be comparable in terms of weight, rate of weight gain, food intake and period of fasting prior to tests have been divided into two groups, control and tumor-bearing. At appropriate intervals following inoculation of the tumor the animals have been studied with regard to their glucose metabolism by the I.V. injection of uniformly labeled C^{14}-glucose. In a series of 15 tumor-bearing animals and ten control animals the rate of disappearance of C^{14}-labeled glucose has been studied. In

addition, the rate of glycogen formation from C^{14}-labeled glucose, the rate of oxidation of glucose to respiratory CO_2, and the rate of conversion of glucose to fatty acid are being evaluated in these tumor-bearing animals.

b) Studies of the biosynthetic pathways of glucose of glycogen and ribose of nucleic acid in liver, skeletal muscle and ascites tumor cells - Studies by previous workers have established that there are several potential pathways of pentose biosynthesis. In cell-free systems it has been shown that ribose phosphate may be synthesized by the oxidative decarboxylation of hexose-phosphate or by a series of reversible non-oxidative reactions catalyzed by the enzymes, transketolase and transaldolase. These two series of reactions may be distinguished by the pattern of C^{14}-labeling present in the ribose derived from specifically labeled precursors. Thus, if ribose phosphate is synthesized primarily by decarboxylation of glucose phosphate, the pattern of C^{14}-labeling of ribose should correspond to the labeling in positions 2 to 6 of glucose. If, on the other hand, ribose-phosphate synthesis proceeds primarily by the series of reactions catalyzed by transketolase and transaldolase, the predicted tracer pattern of the pentose would be quite different. It has been previously demonstrated, in studies by Drs. Paul Marks and Philip Feigelson, that when glucose-2-C^{14} was employed as a source of tracer, ribonucleic acid ribose was labeled primarily in position 2, with a lesser but significant degree of labeling in ribose carbon atom 1. In the glucose of glycogen isolated from these experiments, the tracer was randomized from position 2 primarily to position 1 and to a lesser extent, positions 3 through 6. These findings were interpreted as being compatible with the hypothesis that the ribose of ribonucleic acid was synthesized by a combination of the reactions catalyzed by transketolase and transaldolase as well as by the reaction involving the oxidative decarboxylation of glucose-6-phosphate. The observed

patterns of labeling of the glycogen glucose and the ribonucleic acid ribose indicated that a rapid *in vivo* equilibration between the pentose and hexose pools existed and that, in addition to the Embden-Meyerhof pathway, pentose conversion to glucose via the transketolase reaction appeared to play a role in the formation of the glucose of glycogen even when free glucose was the glycogen precursor.

During the past year these studies have been extended to an investigation of the pathways of synthesis of glucose in the glycogen of skeletal muscle of normal fed and fasted rats *in vivo*. In addition, the *in vivo* synthesis of the ribose of ribonucleic acid of the ascites tumor cell and of the liver of animals bearing the Yoshida ascites tumor has been studied.

In these studies 2-C^{14}-glucose has been used as a source of isotope, and the glucose of glycogen and the ribose of ribonucleic acid has been isolated from the various tissues, namely skeletal muscle, liver, and tumor ascites cells, and degraded by bacterial fermentation. In the case of the degradation of glucose, *Leuconostoc mesenteroides* has been employed for the fermentative degradation. For the purposes of ribose degradation, the organism *Lactobacillus pentosus* has been employed. In the experiments with normal rats, the glucose of liver glycogen was predominantly labeled in position 2. An appreciable migration from C2 to C1 was observed, with a lesser degree of randomization to the other carbon positions of glucose. Fasting was associated with an increase in the specific activity of position 1 relative to position 2 of the glucose of liver glycogen.

In the glucose of the skeletal muscle glycogen from fasted normal rats tracer was present essentially only in position 2. In the study with fed animals somewhat greater randomization occurred, with C1 being relatively more heavily labeled than C3 to C6.

Interpretation of these results in terms of the known alternative routes of glucose utilization has led to the following conclusions:

In liver, the pentose phosphate pathway contributes to the formation of glycogen glucose and this contribution is more apparent in fasted than in fed animals. In contrast, the glycogen of skeletal muscle appears to be derived almost entirely from glucose by direct incorporation of the intact administered 6-carbon unit, both in the fed and fasted state.

The studies of the in vivo pathways of pentose phosphate synthesis in the liver and the tumor of rats bearing the Yoshida ascites tumor, employing glucose-2-C^{14} as the isotopic precursor,have yielded the following results: The liver ribonucleic acid ribose presented an isotopic distribution pattern that was conspicuously different from that of the control rat bearing no tumor. Whereas the bulk of radioactivity in the ribose of control animals was in carbon atom 2, with considerable activity in carbon atom 1 and little or no activity in ribose carbon atoms 3, 4 and 5, the radioactivity of the liver ribose of the tumor-bearing rats was found to be widely dispersed throughout the ribose molecules. In the tumor cells, however, ribose carbon atom 2 exhibited the highest specific activity, the remaining activity being distributed essentially equally through the rest of the ribose molecule in carbon atoms 1, 3, 4 and 5.

The distribution of C^{14}-labeling in liver RNA ribose of the Yoshida tumor-bearing rat thus was in sharp contrast to the labeling pattern previously observed in the normal rat liver RNA ribose. This alteration in labeling pattern may be subject to two alternative interpretations. (1) The tumor exerts an influence on liver metabolism or (2) the observed pattern of ribose labeling in the liver results from normal pentose synthesis by the liver, utilizing, however, a hexose precursor in which all carbon atoms are

labeled as a result of the activity of the tumor itself. This latter alternative is supported by certain recent findings that indicated that glucose-2-C^{14} administered to tumor-bearing mice resulted in liver glycogen containing C^{14} distributed throughout all carbons of the hexose. These findings have been interpreted as indicating that tumor may cause an extremely active reversible conversion of the administered C^{14}-glucose to glucose of glycogen via the Embden-Meyerhof and tricarboxylic acid cycles, resulting in migration of C^{14} from its original position to the other locations in the glucose molecules. Whether these alterations are due to the tumor or to the indirect influence of the presence of the tumor on the metabolism of other tissues is a problem currently under investigation.

c) Relationship between human erythrocyte aging in vivo and the activities of glucose-6-phosphate and 6-phosphogluconic dehydrogenases - Erythrocytes derive most of their energy from glucose utilization. It is well established that the pentose phosphate pathway in addition to anaerobic glycolysis plays an important role in red blood cell metabolism. Little is known however about the changes occurring in carbohydrate enzyme levels associated with red blood cell aging in vivo.

Pentose phosphate pathway activity in red blood cells has been studied by evaluating the levels of activity of the two enzymes catalyzing the oxidative reactions of this pathway, namely glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase.

In the present study the level of both of these enzymes has been found to be elevated in individuals with reticulocytosis. In 49 normal subjects the average level of glucose-6-phosphate dehydrogenase was 14.6 units per gram of hemoglobin, compared with a value of 25.6 units per gram of hemoglobin for the group of patients with reticulocytosis. The average level of 6-phospho-

gluconic dehydrogenase in 40 normal individuals was 19.1 units per gram of hemoglobin compared with 41 units per gram of hemoglobin in the patients with reticulocytosis. The patients studied with reticulocytosis had a wide variety of conditions such as hemolytic anemias, pernicious anemia and neoplastic diseases.

To determine whether alterations occur in the level of glucose-6-phosphate and 6-phosphogluconic dehydrogenase in erythrocytes beyond the reticulocyte stage as they age in vivo, advantage has been taken of certain differences in the characteristics of young relative to old erythrocytes. These characteristics are the lower osmotic fragility and the lower density of the young cells. In the present studies osmotic hemolysis and centrifugation have been employed to fractionate cells according to age and these techniques have been validated by iron-59 determinations performed on blood drawn at intervals following administration of this isotope. It has been found that both glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase are present in much higher concentrations in young mature erythrocytes than in old mature erythrocytes. Evidence has also been obtained that the level of activity of these dehydrogenases parallels the mean cell age of the hemolyzed fraction of red blood cells.

These present findings lead us to the following conclusions:

1. The levels of two enzymes important in the energy metabolism of red blood cells, namely glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase, are high in young mature erythrocytes and these levels diminish in association with the aging of the red blood cells in vivo.

2. Increased levels of these enzymes may be a more sensitive indication of young cell population than the reticulocyte count.

Investigations are under way to evaluate the possible relationship

between aging of the red blood cells in vivo and the level of activity of other enzymes involved in the metabolism of the red cells.

Hematology

a) Pathogenesis of anemia in patients and laboratory animals with malignant disease - During the past year chromium-51 red cell life spans have been performed by Drs. George Hyman and John Ultmann both in conjunction with laboratory investigation and in certain diagnostic problems. The red cell survival has been studied sequentially in patients both prior to and after the extirpation of local tumors and in leukemic patients before and after complete remissions in order to evaluate the effect of therapy on the red cell life spans. In addition, work has been completed with certain animal tumors. In these animals the following has so far been discovered. In rabbits bearing two types of tumors implanted either locally or given intravenously so as to be widely disseminated, there was no abnormality in the red cell life span as measured by chromium-51. Because of this, we turned to another animal system, namely, rats, and using two tumors we found that although there is an apparent shortening of the red cell life span, the entire loss of red cells labeled with chromium-51 can be easily accounted for by hemorrhage either into the tumor, or by red cell diapedesis into the ascitic fluid in animals bearing ascites tumors. Because of this, further efforts are being made to discover a different tumor host system in which red cell life spans and the effect of various chemotherapeutic agents can be correlated.

During the past year we have studied 5 patients with polycythemia vera in remission with particular emphasis on iron-binding capacity, iron-59 disappearance curve, red cell production rates and life span measurements with iron-59 and chromium-51 respectively. From a study of these 5 patients, the following is apparent: (1) A number of these patients, following

phlebotomies over the years, have become severely iron-deficient as evidenced by low iron contents and very high latent iron-binding capacities; (2) in these patients the T/2-Fe^{59} disappearance curve is markedly shortened, in one patient to as little as 23 min. (normal about 60 to 120 minutes); (3) when an adequately absorbed intramuscular preparation of iron is administered and plasma iron saturation is returned to normal, the plasma iron disappearance time reverts to normal. Because of this finding, we feel that a study of the plasma Fe^{59} disappearance rate cannot be used as a key to bone marrow activity in these patients and possibly in other conditions as well. These studies will be continued. The Fe^{59} reappearance in red cells was measured in 3 patients and was found to be essentially normal with the high point reached on about the 13th day. The red cell life span, as measured by chromium-51, was essentially normal in 2 patients, but in one patient it actually was slightly shortened, the 10% level being reached approximately after 65 days.

In an attempt further to delineate the biochemical lesion which might be responsible for the shortened red cell life span in tumor patients, and to discover why there was no reticulocytosis in patients with hemolysis proven by chromium-51 technique and active bone marrow as demonstrated by direct examination or Fe^{59} studies, we studied the glycolysis of whole blood. It was found that the rate of erythrocyte glycolysis of blood from normal volunteers was 12 mg% of glucose consumed per hour with 5 million red cells per cubic mm. The mean glycolytic rate of tumor patients was 14.5 mgms.% of glucose consumed per hour and that of patients with other chronic diseases, 17.4 mg.% The elevated red cell glycolytic rate of patients with tumors and other chronic diseases was statistically significantly greater than that of normals, in the absence of reticulocytosis. This suggests to us that the red cell population of the patients with neoplastic and chronic diseases might be younger than that

of normal subjects and that only some of the young red cells had demonstrable reticulum. In a limited number of patients with bloods having elevated glycolytic rate, chromium-51 red cell life spans were also determined. Of 12 patients, 8 had elevated glycolytic rates as well as significantly shortened red cell life spans. Two patients had normal glycolytic rates and essentially normal red cell life spans. In two patients no correlation between the two tests could be made.

b) Megaloblastic anemia - In the past year cobalt-60 labeled vitamin B-12 has become a routine diagnostic tool in the evaluation of certain patients with anemia. It might be stated parenthetically here that four patients have been admitted to this hospital during the past year as terminal patients with severe anemia, hepatomegaly, and two instances, ascites, and have turned out to have easily curable megaloblastic anemia. Studies in these patients with cobalt-60 vitamin B-12 have helped in each instance.

c) Other studies - Dr. Hyman, in collaboration with Drs. C. Yahia and L. L. Phillips of the Department of Obstetrics and Gynecology of Columbia University College of Physicians and Surgeons, summarized the Delafield-Columbia-Presbyterian Medical Center experience with acute leukemia in pregnancy and reviewed the world literature in order to outline best the optimal current therapeutic program.

Dr. Hyman, working with Dr. I. Jaffe, described a case of hematologic toxicity with demicolcine (colcemide) simulating acute leukemia and summarized current hematologic toxicity of this agent, concluding that its dangers outweighed its benefits.

Myeloma

The laboratory of Dr. Elliott Osserman continues to pursue its studies

of multiple myeloma. This program is now in its fourth year.

Its aim has been to provide a better understanding of the natural history and clinico-pathological variants of this cancer, and concurrently to obtain further knowledge concerning the nature of the unique abnormalities in protein metabolism associated with this disease. For purposes of presentation, these studies may be divided into two sections: a) the clinico-pathological spectrum of myeloma - - clinical investigations of patients with this disease; and b) experimental myeloma: studies with the transplantable mouse plasmacytoma.

a) The clinico-pathological spectrum of myeloma - In routine clinical studies, the filter paper modification of the electrophoretic technique has been employed for the study of the serum and urinary proteins. Studies have been performed on the majority of patients seen on the Medical Service of the Francis Delafield Hospital and the majority of patients at the Presbyterian Hospital in whom multiple myeloma was considered as a diagnostic possibility, or in whom this diagnosis had been established by other laboratory tests. Also included in these studies have been many patients with unexplained anemia, hyperglobulinemia, elevated sedimentation rate and proteinuria.

It is impossible to present in this limited space a comprehensive account of the observations on these patients. A few of the more important features, however, may be noted:

1. Multiple myeloma is not a rare disease. One hundred and fifty cases have been assembled in this series over the course of the past three and one-half years.

2. Characteristic serum and/or urinary protein abnormalities may be demonstrable for many months and possibly years before the appearance of clinically recognizable symptoms or signs of myeloma. Twelve such asymptomatic

patients have been followed for periods ranging up to three years. Eight of the twelve have subsequently exhibited widespread osseous destruction characteristic of advanced myeloma. Presumably, in this asymptomatic, "pre-myelomatous" phase, there exist widely scattered groups of abnormal plasma cells, producing their abnormal protein by-products, but not present in aggregates of sufficient size to produce radiographically or symptomatically evident disease. The documentation of this early, asymptomatic phase forces a revision of existing concepts of the natural history of myeloma.

3. The clinico-pathological features of individual cases of myeloma display a striking degree of variability. The range of this spectrum of clinical patterns extends from those cases with predominantly osseous pathology due to plasma cell tumors, described above, to individuals whose major symptoms and signs are referable to the deposition of abnormal proteinaceous material in selected tissue sites (para-amyloidosis); at the other end of the spectrum are patients with the characteristic clinical and histopathological features of disseminated lymphosarcoma whose serum and/or urine are found on electrophoretic analysis to contain abnormal myeloma proteins. Justification for postulating the existence of this continuous spectrum of disease patterns is provided by documentation of the "intermediate" cases, i.e. cases with intermixed features of "bony myeloma", "lympho-myeloma" or "para-amyloid myeloma".

4. The natural history and clinical course of individual patients with myeloma is equally as variable as the clinico-pathological features which each displays. The complexity of the problem is apparent from the recognition that an individual case may succumb to his disease in a time period renging from a few weeks to over twenty years from the onset of symptoms referable to myeloma. Documentation of the occurrence of "spontaneous" remissions in symptoms,signs and biochemical findings has introduced further

complexity into this already complex situation. Long-term clinical and biochemical studies are in progress on a group of over one hundred cases in an endeavor to define some of the determinants of the clinical course. Serial quantitation of the abnormal serum and/or urinary proteins in myeloma currently represents one of the best objective criteria for the evaluation of the efficacy of a given therapeutic regimen in a disseminated neoplastic disease.

5. Coincident with the over-production of the abnormal proteins of myeloma there develops a deficiency in the quantity of normal serum γ-globulin. As a result, at least in part, of this deficiency, the myeloma patient displays an inordinate increase in susceptibility to pneumonia and other bacterial infections. In order to define further the extent of alterations in host responsiveness to "antigenic stress" in this disease, the homograft reaction in myeloma has been investigated. In a single subject with myeloma, full-thickness skin grafts from another adult subject have shown complete tolerance with no evident inflammatory reaction or rejection after 16 weeks (at this writing). The normal homograft reaction would appear to be markedly impaired in patients with this disease.

b) Experimental myeloma: studies with the transplantable mouse plasmacytoma - Within the past year, a transplantable plasma cell tumor has been described in C_3H mice. Animals bearing this tumor develop serum protein abnormalities closely paralleling the findings in the sera of human myeloma subjects. This experimental tool has been available to this laboratory for the past 9 months. The following observations have been made to date:

1. The serum concentration of abnormal protein is proportional to tumor size. Serial electrophoretic examination of the serum of the tumor-bearing animals has demonstrated a progressive increase in the abnormal serum peak with tumor growth. A technique for the electrophoretic separation and

quantitation of the serum proteins in a single drop of tail vein blood has been developed.

2. Surgical excision of localized tumors results in a disappearance of the abnormal protein from the serum. Because this tumor is a well-differentiated, slow-growing and late-metastasizing neoplasm, "cure" by surgical extirpation is possible. Serial study of the serum proteins following tumor removal provides a means for determining the metabolic degradation rate of this abnormal globulin. The half-life has been found to be 3-3.5 days. Knowing the size of the tumor removed, the half-life of the protein, and estimating the volume of distribution of the protein it can be calculated that 25-35 mgm. of abnormal protein are produced per gram of tumor per day.

3. The abnormal serum globulin is immunologically distinct from the normal γ-globulin of the mouse. Rabbit antiserum to the abnormal protein (isolated by continuous-flow preparative electrophoresis) shows some cross-reaction with normal mouse serum. After absorption of all the cross-reacting antibody to normal serum, however, a strong precipitin reaction is still obtained with the homologous antigen (i.e. the abnormal globulin). This has been clearly confirmed by the Ouchterlony agar-diffusion technique.

4. The plasma cells of this tumor can be propagated in tissue culture, and evidence has been obtained for the continued elaboration of the abnormal protein by these cells grown *in vitro*. This work is still in its preliminary stages, but it may be stated that following periods of up to three weeks of *in vitro* cultivation, with bi-weekly changes in the tissue culture media, the abnormal protein can still be demonstrated in the feeding fluid by immuno-chemical methods. This technique should provide a useful tool for the study of the mechanisms of protein synthesis.

Cardio-pulmonary physiology

The Department of Cardiology was expanded during the last academic year to include a cardio-pulmonary laboratory under the direction of Dr. Henry Heinemann. Drs. Heinemann and C. Emircil have initiated the following investigations:

a) The effect of CO_2 on the osmotic activity of mixed venous and arterial plasma - Previous observations by Moschia and Barron suggest that CO_2 contributes to the measurable total osmotic pressure determined by freezing point depression.

The electrolyte concentration, CO_2 content and freezing point depression were determined in peripheral arterial, peripheral venous and mixed venous blood obtained during cardiac catheterization. Determinations were also done after hyperventilation and following *in vitro* incubation of whole blood with gas mixtures of varying pCO_2. It was found that CO_2, mainly carried in plasma as HCO_3, contributes to the total solute content as measured by freezing point depression, but that *in vivo* at rest the A - V difference is too small to be accurately measurable. It is furthermore concluded that the accumulation of HCO_3 in plasma is in part offset by the chloride shift and that in addition to the accumulation of CO_2, changes in pH, ionic strength and intracellular and extracellular cation binding contribute to the difference in freezing point depression between arterial and venous plasma samples.

b) Reflex regulation of renal excretion of water and electrolytes - Evidence is available to suggest that negative pressure breathing both in dogs and man leads to a water diuresis (Siekert, Gauer). This has led to the assumption that negative pressure breathing causes intrathoracic pooling of blood and subsequent diuresis via an unexplained homeostatic mechanism

(volume receptors ?). Since pooling of blood in a limited area such as the chest cage may lead to distension of vessels, it was thought that stretching of these vessels alone without changing blood volume, cardiac output, or pressure may initiate the same response. A catheter was designed to permit distension of the superior and inferior vena cava without obstructing blood flow. Glomerular filtration, water and electrolyte output by the kidneys were measured before, during and after distension of the superior vena cava in 2 dogs during 4 experiments.

The results so far obtained do not suggest that there is an area in the superior vena cava sensitive to stretch and involving the regulation of water and electrolyte excretion.

c) Effect of mercuhydrin on the excretion of "free" water - Previous observations in patients with diabetes insipidus indicate that mercuhydrin increases the excretion of "free" water during water diuresis, in addition to its effect on solute (Na and Cl) output. This strongly suggests that mercuhydrin acts proximal to the site of "free" water formation. To establish if mercuhydrin acts quantitatively differently during H_2O diuresis and "anti"-diuresis, excretion of "free" water, Na and Cl was determined in 2 patients admitted to the Metabolic Ward. The studies were done twice, once during maximal water diuresis and once during water deprivation. There was no quantitative difference in Na and Cl excretion under both test conditions, but the excretion of "free" water increased during water diuresis while active water reabsorption (T^cH_2O) increased during water deprivation.

d) Hyponatremia in a patient with an intra-pulmonary metastases from breast carcinoma - This interesting case was studied in collaboration with Dr. J. Laragh. It was found that the defect was characterized by

hyponatremia, excessive Na and Cl loss in the urine and continuous excessive water reabsorption. Despite large fluid loads (hypotonic) the patient was unable to produce urine hypotonic to the plasma, i.e. extracellular fluid.

Clinical chemotherapy

The formal evaluations of a number of compounds mentioned in last year's report were completed and studies on several new drugs were initiated.

Chlorambucil - Chlorambucil, p-(di-2-chloroethyl)aminophenylbutyric acid (CB 1348), an aromatic nitrogen mustard, was administered by mouth in doses ranging from 2-30 mg daily for an average of 8 weeks. In 60 patients the average course amounted to 435 mg, or 7.1 mg/kg. In the largest group, 32 patients with chronic lymphocytic leukemia given 36 courses of treatment, significant objective improvement occurred in 21 instances lasting an average of 9 months. Of 14 patients with lymphocytic lymphosarcoma, 7 showed significant improvement, but only with more prolonged and more intensive administration of chlorambucil. In Hodgkin's disease (8 patients), the results were much less encouraging, while there was no effect in six patients with multiple myeloma. In the dosages used, chlorambucil was practically without side-effects; in fact, the drug appeared of particular usefulness in the 14 patients with chronic lymphocytic leukemia who had moderate thrombocytopenia without clinical manifestations of bleeding prior to therapy as compared to nitrogen mustard and TEM.

Thio-TEPA - The effects of 112 therapeutic courses of triethylenethiophosphoramide (N-N'-N''-triethylenethiophosphoramide, S-TEPA, Thio-TEPA) in 100 patients with various malignant neoplasms have been studied. The average course was 50 mg. given intravenously over 5 - 7 days. Significant objective changes were obtained in 19 of 49 courses given to 43 patients with malignant lymphomas, but these changes were generally of short duration. In 57 patients

with various carcinomas and with malignant melanoma, significant objective improvement was observed only in those with ovarian carcinoma. Fourteen of 27 patients with the latter tumor were objectively benefited and the survival time of these was significantly prolonged when compared with the patients in whom the therapy failed. The delayed hematologic depression occurring in 43 instances and clinical manifestations of toxicity in 17 patients, and the unpredictability of the dosage-toxicity relationship indicate the low therapeutic value of thio-TEPA, except in ovarian carcinoma.

Triethylene melamine - Dr. George Hyman, in collaboration with Dr. Algernon B. Reese of the Institute of Ophthalmology, reported on results of combination therapy of retinoblastoma with triethylene melamine and radiotherapy. Because of the possibility of synergism between radiotherapy and triethylene melamine therapy, a study was commenced in January, 1953, at the Institute of Ophthalmology and the Columbia-Presbyterian Medical Center in which 50 children with retinoblastoma received combined therapy. This combination appeared to be both feasible and apparently successful in arresting retinoblastoma in a significant proportion of children with early disease. It was found that triethylene melamine could be administered safely to children in doses proportionately larger than those given to adults and that the route of choice was intramuscular.

2-[Di-(2-chloroethyl)aminomethyl]benzimidazole - This benzimidazole mustard has been administered during the past year to 12 patients by the intravenous route and to 2 patients orally. Preliminary studies in man showed that in an occasional patient nausea occurred and in one patient a severe seizure. In doses of 2 mgms/kg given daily over 4 days a depression of the hematopoietic system with leucopenia and thrombopenia was noted in most patients after a period of one to two weeks. To date no therapeutic

results have been observed in any of these patients.

5-Fluorouracil - This compound has been administered to date to 12 patients with a variety of neoplasms by the intravenous route. Preliminary results would indicate that this drug, in doses of approximately 10mg/kg/day x 5 days, produced a significant fall in the platelet count in about 7 to 14 days in about half of the patients. In some of these patients the platelet depression was accompanied by mild leucopenia. In none of the patients was there clinical toxicity secondary to the marrow depression, and the hemogram returned to normal within about 10 days. There have been no therapeutic benefits noted to date in any of these patients.

CB-3025 (Phenylalanine mustard) - Phenylalanine mustard has been administered to date to 3 patients by the oral route. In none of these patients was a therapeutic effect on his malignant melanoma noted. In one patient, a total dose of 4.2 mg/kg over a 33 day period was followed by severe hematologic depression with a white count of 500 and platelet count of 40,000 but the patient's bone marrow had recovered fully 3 weeks later.

Neurological Surgery

Professor J. Lawrence Pool and Dr. Carlos Cortelazzi have completed a clinical evaluation of the possible therapeutic effect of the antimetabolite, 8-azaguanine, in patients with glial tumors. The substance has been administered to 11 patients by intravenous route and to 5 patients by intracarotid infusion at the Neurological Institute of New York and the Francis Delafield Hospital.

The use of 8-azaguanine is based upon the earlier studies of Dr. Margaret Murray of the Laboratory for Cell Physiology showing that it is capable of arresting the growth of glioblastoma cells in tissue culture. The drug has been used extensively elsewhere in attempting to alter the cause of various malignant diseases.

Intravenous administration of a sterile saline solution of 8-azaguanine was carried out in 11 patients. All patients had histological verification of the diagnosis of glioblastoma multiforme. These patients received 8-azaguanine alone, and 8 patients received simultaneous radiotherapy and chemotherapy.

Survival time after operation was not prolonged by either 8-azaguanine alone, or by radiotherapy plus 8-azaguanine, as shown in Table 1.

TABLE 1

	CONTROL	8-AZAGUANINE TREATED
Number of patients	55	10
Surgery and Radiotherapy	43	8
Surgery without Radiotherapy	12	2
Average survival period (Surgery and Radiotherapy)	7.5 mos.	6.8 mos.
Average survival time (Surgery without Radiotherapy)	7.6 mos.	5.0 mos.

Dosage of 8-azaguanine was 100-250 mgm per patient per day as long as tolerated. Total dose ranged from 0.6 to 2.0 grams. Treatment was terminated by the appearance of toxic symptoms. These are summarized in Table II.

TABLE II

REACTIONS OF INTOLERANCE

(Demonstrating the pathological manifestations attributable to 8-azaguanine)

NAME	UNIT NUMBER	RASH	PHLEBITIS	VOMITING	DIARRHEA	LEUCOPENIA	DROWSINESS	TENESMUS
M.R.	022979	+	0	+	+	0	0	0
P.G.	002758	+	+	0	+	0	0	0
W.M.	134665	+	0	+	0	0	+	0
B.W.	135009	0	0	0	0	0	0	0
N.M.	112962 FDH2732	+	+	+	0	0	0	0
W.G.	095424	+	+	0	0	0	0	0
D.E.	829595	0	0	0	0	0	0	0
S.M.	066106	+	0	0	0	+	0	0
W.J.	041721	+ itching	0	0	0	0	+	+
B.H.	FDH192	0	0	+	+	0	0	0
F.J.	FDH1531	0	0	0	+	0	0	0
TOTAL		7	3	4	4	1	2	1

Body rash was the most common symptom, characterized by pink maculae and papulae with a tendency to concur, starting in most cases in the trunk, the tendency of their extension being variable. In the severe cases it was followed by desquamation of the skin. In only one case was the rash accompanied by pruritus.

Intracarotid infusion was carried out in 5 patients with verified glioblastoma multiforme. Three patients received intracarotid 8-azaguanine alone, and 2 patients were given 8-azaguanine during a course of radiotherapy.

Dosage of 8-azaguanine, administered as a continuous infusion of 100 mgm per liter, ranged from 0.4 to 2.0 gms. per patient.

Survival time was not prolonged in any of these 5 cases.

Treatment was discontinued in each case because of toxic reaction. Intracarotid infusion produced localized toxic symptoms consisting of rash over the homolateral side of the face and neck, and continuous, excessive salivation. The latter was most distressing to the patient and was only partially controlled by atropine sulfate.

The clinical impression gained by these studies may be summarized by saying that while life was not prolonged by administration of 8-azaguanine to a series of patients with glioblastoma multiforme, it was of interest that definite clinical improvement occurred in 7 of the 11 cases treated by intravenous route and in 3 of the 5 patients who received the drug by intracarotid route. Brief, temporary improvement consisted of markedly reduced tension of a subtemporal decompression in one patient, and mental clearing and improvement in hemiparetic and aphasic symptoms in the other 9 patients. Interestingly this improvement occurred only after 48 hours of treatment by either route and lasted for only 4 to 14 days. Since this transient improvement persisted beyond the period of treatment in most cases, it does not appear that it is a non-specific effect or dehydration response.

Efforts to protect the patient from the toxic effects of 8-azaguanine by administration of vitamin C, pyribenzamine, or cortisone were futile.

In related animal studies, experiments in the use of this drug in mice,

rabbits and dogs were carried out prior to the human administration. CH_3 mice with verified tumors resembling glioblastoma were tested. The tumors were induced by implantation in the brain of methylcholanthrene. This work, carried out by Dr. Frederick Boykin, did not demonstrate statistically significant prolongation of life or reduction of tumor size in the tumor mice. However, in some of these animals there was definite reduction in the rate of tumor growth and evidence of histological changes in the induced tumors, suggesting arrest of cell growth by the use of 8-azaguanine. The drug was given to these animals by the intraperitoneal route, in dosages of 50 mgs. per kilo. In other words, in some instances the drug seemed to be effective in slowing the rate of tumor growth.

The intracarotid infusion technique was tested on rabbits and dogs in dosages equivalent to that later used for treatment in man. There were no untoward effects unless a greater concentration than that used clinically was given, which then led to cerebral edema on the injected side of the brain.

From our laboratory and clinical experience, we feel that in some cases 8-azaguanine may exert some slight effects on glioblastoma multiforme tumors in mouse and man. The fact that this drug is not more effective could be attributed to (1) an insufficient concentration of the drug reaching the tumor because the substance is broken down so rapidly after injection, and (2) the difficulty in giving a sufficiently concentrated dose because of toxic effects. For example, the tolerated clinical dosage does not even closely approach the effective concentration used in tissue culture studies.

This report on the use of 8-azaguanine is offered to save others from duplicating such a study, and to indicate that intracarotid injections of chemotherapeutic agents are feasible and need not lead to the serious cerebral complications reported in the literature following the use of a more

toxic substance, nitrogen mustard.

There is a definite feeling that successful treatment of glioblastoma multiforme and other brain tumors will ultimately depend on intravenous or intracarotid infusion of tumoricidal agents, probably in the form of anti-metabolites.

In additional activities, Dr. Edward Schlesinger and Dr. Juan Taveras are engaged in a study of intracranial localization with radioisotopes which has been greatly improved by simplifying the method of recording to enhance clinical application. Collimation methods are now standard and no longer require special investigation as a separate problem.

Dr. William Clark, our collaborating physical chemist, has finished preliminary studies and chosen what he considers to be the likeliest carriers for radioactive isotopes. He now plans to synthesize them for clinical trial.

Pathology

Dr. Theophilus M. Roberts has been studying the histologic changes in mice exposed to high altitude conditions (low oxygen tensions). Under the direction of Dr. Alvan Barach and Dr. Hylan Bickerman, mice with and without induced carcinoma (C755) were exposed for various periods of time. Dr. Roberts compared the body weights and tumor weights of the various groups. Histologic study of all organs and of tumors utilizing special vascular stains and micrometer measurement of vasculature was carried out.

Dr. John T. Gorman has been studying the extra-skeletal mixed tumors with Dr. A. Purdy Stout. The study has already brought to light some hitherto undescribed unusual features.

Dr. A. Purdy Stout has continued his investigations of the changes in the bronchial mucosa in connection with cigarette smoking. This is a continuation of a program carried out by a group headed by Dr. Oscar Auerbach at the East Orange V. A. Hospital.

Dr. ... S. Oppenheimer and his associates, with Dr. Stout, are continuing work on the general problem of the significance of sarcomas developing in relation to plastic films, with special reference to the mechanisms of their production and the precancerous stage.

Dr. William T. Hill and Dr. Stout have reviewed the leiomyosarcomas in the soft tissues. The work is now approaching completion.

Dr. Hill and Dr. Henry Azar collaborated with Dr. E. Osserman and completed a study of malignant lymphomatous conditions in which a myelomatous type of serum protein was demonstrated. Use of pyronine stains showed that the cytoplasm of these cells was more pyroninophilic despite the absence of other stigmata of myeloma cells.

Dr. Silvio Fiala, Mrs. Anne Fiala and Dr. Edith Sproul reported to

the American Assocn. for Cancer Research and the Federation of Biological Societies completed studies on the localization of carcinogens and the relationship of the resulting altered metabolism to the changed distribution of cytoplasmic ribonucleic acid. Protein-bound carcinogen was found to accumulate mainly in the soluble phase and secondly in the microsome fraction. The absence of mitochondrial binding of carcinogen refuted the theory of direct inhibition of respiration by carcinogens. The gradual depletion of mitochondria and of respiratory enzymes paralleled the depletion of cytoplasmic microsomal-bound RNA during carcinogenesis. As macromolecular RNA increased, a proportional increase in glycolytic enzyme activity occurred. The cytoplasmic events during carcinogenesis were contrasted with those of hypertrophy and hyperplasia in target organ cells following stimulation by anterior pituitary hormones. Both patterns of change demonstrated a relationship between formation of respiratory enzymes and synthesis of microsomal-bound RNA.

Dr. Edith Sproul has studied the sputa of well individuals, and of patients with and without malignant disease of the lung. These specimens were obtained by Dr. Hylan Bickerman and Dr. Alvan Barach by administration of hypertonic saline in tergemist. There was good correlation between the lung condition and the diagnoses on the induced sputum. This study was the initial work in a proposed screening program for lung cancer among individuals not producing sputum.

Dr. Diane W. Crocker has been reviewing sections of familial hypertrophy of mucous membranes in conjunction with Dr. William Kutscher and Dr. John Firo.

Dr. Prapont Pivarath, Dr. William Hill and Dr. Stout have completed a report on the rare leiomyosarcomas of the esophagus.

Pediatrics

Dr. James A. Wolff with Drs. Frederick H. von Hofe, William E. Homan and Calvin Tribiano has continued studies on the chemotherapy of acute and subacute leukemia in children in cooperation with the Clinical Panel of the Cancer Chemotherapy National Service Center. An evaluation was made of the effectiveness of 6-mercaptopurine when used in combination with azaserine as compared with 6-mercaptopurine alone. The results of this study are now being evaluated statistically and it would appear that the single drug is therapeutically as effective as the combination of drugs. There is some indication that the length of remission produced may be somewhat longer with the combination than with mercaptopurine alone.

At the present time a new drug, DON, is being evaluated in combination with 6-mercaptopurine for comparison with 6-mercaptopurine alone in standard and in greater than standard dosage.

Radiological Research Laboratory

As in previous years, the activities of the Radiobiological Research Laboratory have been divided between a research project for the Atomic Energy Commission and problems related to therapeutic radiology and medical uses of radioactive isotopes. While the work carried out for the AEC is concerned with problems of special interest to the Commission, it generally involves fundamental problems in radiological physics and radiobiology.

The radiological physics section is primarily concerned with absolute measurements of both the quantity and quality of radiation as delivered to tissue. In addition to investigating new methods of such measurements, considerable effort is being spent in determining certain parameters which are essential in the precise dose measurement involving the ionization method. Radiobiology under the Atomic Energy Commission contract deals largely with problems related to radiation protection. Below is a brief summary of the activities.

Dr. G. Failla, Mr. W. Gross, Mr. B. Allen and associates

Radiological Physics: Measurements of tissue doses produced by radioactive isotopes variously distributed have been continued. An accurate determination of W, the average energy lost by S^{35} beta rays per ion pair produced in air, has been made. This value is 33.6 $\pm$ 0.3 ev. and is in good agreement with the value determined later at the Bureau of Standards by a different method. The value of W for water vapor relative to air for the same beta rays (0.894) has been determined experimentally for the first time. A preliminary value (1.23) of the specific energy absorption of water with respect to air for the beta rays of Ni^{63} has been obtained. A new method intended primarily for determining the disintegration rate of K-capture isotopes from their gamma rays has been tested with Au^{198}. The results

agree within ± 3% with those obtained by the Bureau of Standards using a different method.

For the measurement of tissue doses of low voltage x-rays it is necessary to have ionization chambers with walls of a conducting material having an atomic composition very similar to that of the tissue of interest (muscle, bone, fat, etc.). The requirements in this case are more stringent than for chambers intended to measure doses of neutrons. New tissue equivalent conducting plastics are being developed in cooperation with Dr. F. Shonka of St. Procopius College and Dr. J. E. Rose of the Argonne National Laboratory. New types of ionization chambers and electrometers are also being developed. Some new methods for testing the tissue equivalence of the conducting plastics with x-rays and the behavior of insulators are being used.

Dr. H. H. Rossi, Mr. W. Rosenzweig, Dr. R. H. Hill and associates

Radiological Physics: The work on tissue equivalent ionization chambers for neutron dose measurements is passing from the research to the technicological stage. At least one manufacturer intends to market these chambers and consultation was furnished to this manufacturer, as well as to other individuals who were interested in making their own chambers. However, one new model has been developed for protection study. In addition, radiation calibrations primarily involving neutron sources have been done at a number of other laboratories.

Acquisition of a 256 Channel Differential Analyzer has aided greatly in further studies of L.E.T. (linear energy transfer) studies of dose. The L.E.T. distribution of a number of neutron sources has been evaluated, both at this laboratory and at a facility at the Argonne National Laboratory. Preliminary studies have been performed at the Presbyterian Hospital betatron, in which the neutron dose in a stray radiation field was evaluated.

Extensive studies of the properties of the plaque-type mutant T_{1s}, discovered by Dr. Hill last year, have been carried out. This mutant was employed in an extensive study of bacterial mutation. As a result of this work, it would appear that mutation, at least with respect to phage resistance, is a gradual rather than an abrupt process. Because of the fundamental importance of this observation a good deal of further work in this field will be performed in the coming year.

Dr. R. Rugh and associates

Radiobiology: Research is in three major directions: Lethality studies, effect of radiation on the gonads, and effects of radiation on the fetus.

Hibernating and tranquillizing drugs have been used with adult mice to determine their value in altering the LD/50/30 days of whole body x-irradiation. Heterosis (hybrid vigor) has been demonstrated as effective when the lethality data of CF_1 x C_{57} hybrids are compared with data for the pure stocks. An attempt has been made to correlate the degree of lymphopenia following a low level whole body exposure of 50 r (rats) with subsequent tolerance of an LD/50/30 day level of 600 r x-ray exposure. It was hoped that the response of peripheral blood to a low level of exposure might be an indicator to subsequent reactions to higher levels of exposure.

The minimum x-ray exposure of the adult female to cause ultimate sterility has been determined as 20 r. The fertile life span of every female so exposed was reduced. Males of any species appear to be more radiosensitive than the females. Castrated males are more radioresistant than their normal brothers, and these were made still more radioresistant by the injection of estrogens. Even within the 4 1/2 day estrus cycle of the female there is a 2-fold variation in radiosensitivity, with maximum resistance during estrus. At every phase of estrus the female is more radioresistant than is the male.

The following studies are being carried out with regard to effects of radiation on the fetus:

1. The fetal nervous system, particularly the neuroblasts, is extremely radiosensitive. The developing neural retina can be severely damaged by tolerable levels of x-radiation, but by 24 hours the phagocytes begin to clean up the cellular detritus and by 72 hours the fetal retina appears to be quite normal. By 2 months the eye will be definitely microphthalmic and there is no way to determine any effect on visual acuity in the mouse. Apparently the effect of x-irradiation of the fetus is deletion of elements of the nervous system which are actively differentiating at the time of insult. Survivors have defects related more to the time of insult than to the level of x-radiation.

2. The production of sterility in either sex depends both on the dose of radiation and the stage of fetal development. Quantitative data are being obtained.

3. The fetus has neuroblasts at all times and these cells are extremely vulnerable. Rats irradiated in utero with very small doses and allowed to survive to adulthood are being subjected to psychological tests to see if there has been damage. There are no histological or cytological manifestations of radiation effect.

4. The placental barrier between the mother and the fetuses is being used to test whether splenic homogenates exercise their radio-protective effects through cellular components or hormone-like products.

5. X-irradiation of the lactating mouse has been shown to affect the growth of the suckling young only by reducing their food intake; there appears to be no evidence of indirect morphological damage to the young when the mother is exposed.

Dr. H. Rossi, Mr. F. DeFriess and associates

Clinical Radiation Physics: Although betatron operation has on the whole been satisfactory, occasional failures have required modification of some of the circuits in the machine, notably in the injection and expansion cycle. To facilitate patient orientation it is desirable to have optical devices that produce light beams which are either coincident with the main axis of the beam or precisely at right angles. Because of the large size of the betatron room, development of such apparatus was found to be a rather difficult engineering problem. However, an accurate optical back-pointer has been constructed which "tracks" vertical motion of the betatron with the precision of better than 3 mm. A side pointer has been designed and is in the process of construction.

For purposes of radiation economy, it is desirable to have a variable aperture for the betatron. Large amounts of lead are required for adequate shielding and considerable precision in collimation is necessary for good beam definition. A device incorporating these features has been designed and construction is almost complete.

As an outgrowth of radiation safety studies, it has been decided to initiate two experiments designed to study patient dose in diagnostic procedures. One of these will involve a determination of integral dose received under various conditions of kilovoltage, field size, etc. Since this project will be carried out utilizing a new technique which is quite rapid, a large amount of information should be obtained quickly, once the equipment required has been secured. The other study will involve a determination of the dose received at various points in a phantom and the correlation of these figures with the degree of blackening of a photographic emulsion placed behind the phantom. This work is designed to explore the possibility of changes in

technique, which may lead to lessening of patient exposure.

Studies of the absolute output of the betatron have yielded this quantity to a precision of a few per cent. Correlation of these figures with studies of the relative output of most of the other medical betatrons in the U. S. makes it possible to obtain an absolute output for all of these machines. As a result, it will also be possible to arrive at some estimates of the relative biological effectiveness of 24 MV x-radiation.

A number of studies of the physics group were performed in cooperation with various individuals. These include experimental measurements of differential tissue absorption in cooperation with Mrs. Beverly Cohen, water phantom measurements at various qualities for a possible modified isodose system in cooperation with Mr. Carl Braestrup, and calculations and measurements on betatron rotation in cooperation with Dr. M. Kligerman. This last project resulted in a paper which was given at the Radium Society meetings.

As in previous years a considerable amount of routine work was performed, including calibration of therapeutic units, direct measurements and calculation of radium applications, assistance in training residents and treatment planning.

Dr. E. H. Quimby and Mrs. Cohen

Cancer Research Project on Radiation Dosage: The study on effects of radiation quality, target-axis distance, and field size on the efficiency of rotation therapy of lesions in different parts of the body has been completed. A preliminary report on this material was presented at the 8th International Congress of Radiology in Mexico City in July 1956, and a definitive report at the meeting of the American Roentgen Ray Society in Los Angeles in September. At this meeting also Dr. Quimby and Mrs. Cohen presented an exhibit based on the material of this study; this received an Award of Merit.

Dr. G. Failla and Dr. Edith Quimby received the annual national award of the American Cancer Society at the Society's annual meeting in November, for their studies on radiation dosage, carried out over many years.

Radiological

Presbyterian Hospital

The 24 million volt betatron has been in continuous operation for more than eighteen months and is being utilized both for clinical radiotherapy and physical research. Drs. Morton Kligerman, Norah Tapley, and Miss Ginette Jacob have completed a study of the dosimetry of rotation therapy with the betatron.

Dr. Harold Jacox, with Dr. Fred Lucas, is studying the radiation effects on normal and cancerous tissue removed at autopsy and is investigating the clinical results of radiation therapy of carcinoma of the larynx and urethra at this institution. He is writing a chapter on radiation therapy for a forthcoming book on lung and mediastinal tumors.

Dr. Kligerman is continuing his experiments with Dr. Daniel Shapiro of the Department of Surgery on the augmentation of radiotherapeutic effects by multi-combination cancer chemotherapy. The preliminary results of these studies on animals have been very encouraging and hold promise of being an important adjunct to our armamentarium against neoplastic disease. Drs. Kligerman and Tapley are working with Dr. J. J. Jacobson and Dr. Ferdinand McAllister of the Department of Surgery in investigating the effects of adrenal irradiation on experimentally produced ascites in dogs. Drs. Kligerman and Tapley are also continuing their study with Dr. Algernon Reese of the Department of Ophthalmology and Dr. George Hyman of the Department of Medicine in investigating the treatment of retinoblastomas by irradiation alone and in combination with triethylenemelamine. Dr. Kligerman and Dr. Virginia Frantz of the Department of Surgery are continuing research on a study of carcinogenesis from external and internal radiation in the thyroid of the Long-Evans rat.

Francis Delafield Hospital

a) Radiotherapy

Dr. Ruth Guttmann is continuing her clinical evaluation of the results of supervoltage and has been evaluating the comparative results of the treatment of neoplasms with conventional 250 KV irradiation as compared with 2 mev irradiation. She is also studying the effects of irradiation on lymph node metastases. Thus far she has observed no difference in the response to irradiation between metastatic adenocarcinoma and squamous cell carcinoma and no difference in the response of nodes in different areas. Small nodes responded better than larger ones and metastatic nodes could be sterilized by irradiation while there was still persistent disease in the primary lesion.

Dr. Guttmann and Dr. Carl Steglich have re-opened their studies of the effect of supervoltage irradiation on the esophagus. Dr. Guttmann and Dr. Doris Date have completed their studies on pulmonary changes following 2 mev therapy for breast cancer.

Mr. Carl Braestrup, Mr. George Hertsch, and Mr. Richard T. Mooney have completed a study of transit dose measurements for Cobalt 60 Teletherapy.

b) Radiodiagnosis

Dr. Arnold Bachman is continuing his studies on intra-osseous venography with special reference to the azygous system in an attempt to distinguish between benign and malignant tumors and to determine operability. He is also studying the roentgenographic appearance of laryngeal tumors and is investigating the technique of the roentgen detection of colonic polyps.

c) Radiobiology

Dr. Anna Goldfeder is continuing to study the properties of malignant

tumors which govern the rate of growth, metabolic activity and radiosensitivity. She is investigating the significance of the time dose relationship in the biological response to ionizing radiation as well as the mechanisms involved in the biologic effects of ionizing radiation.

Surgery

Cancer research investigations in the Department of Surgery have ranged from the problem of thyroid carcinogenesis from internal and external irradiation through the cultivation of adenovirus in human cancer cells in tissue culture to the further characterization of the criteria for operability and inoperability in cancer of the breast.

Thyroid research

The problem of the possible carcinogenic effect of radiation to the thyroid gland has long been of interest to Dr. Virginia Frantz and her colleagues in the Thyroid Clinic, particularly with the introduction of radioactive iodine as a diagnostic and therapeutic agent. As early as 1949 a report was published of the results of a questionnaire sent out by Drs. E. Quimby and S. Werner from our own clinic to radiologists, internists and surgical pathologists in an effort to find out whether thyroid carcinoma had ever occurred in a previously irradiated gland; no evidence of increased incidence of carcinoma was found. These were, of course, adult patients. In many clinics such as our own a large number of patients were successfully treated for thyrotoxicosis by external therapy. The bulk of these patients were treated between 15 and 25 years ago, so that there has been ample time for carcinoma to become apparent clinically if it had been initiated by radiation damage to these adults. In this clinic approximately 738 patients were treated and in no instance in our cases so treated has a thyroid carcinoma become evident.

Another approach in our clinic has been the review of thyroid cancer patients. We have approximately 466 cases operated upon for carcinoma of the thyroid to date. In 177 of these cases up to December 31, 1956, there is a possible 10-year follow-up. Only one patient gave a history of previous

radiotherapy for Graves' disease. This patient was treated at the age of 34 and her operation for thyroid carcinoma was 13 years later.

The recent article by Simpson and Hempelmann emphasizes the possible direct relationship between irradiation, at least in children, and the development of malignant tumors of the thyroid. There has been enough concern about this so that in some clinics when I-131 is used for the treatment of primary hyperthyroidism a lower age limit has been set for this form of therapy, usually 40 to 45 years. Beyond this age the life expectancy of a patient and the characteristic indolence of growth of the low grade forms of thyroid cancer combine to make the development of the aggressive disease unlikely in the later years of these patients' lives.

A new alarm, however, about possible carcinogenesis was spread by the publication in January 1952 of an article which received very wide immediate attention. This was the second article from Chaikoff's laboratory on the induction of thyroid tumor in rats by a single intraperitoneal dose of radioiodine. Apparently in several institutions there was some scepticism about the validity of the figures in this series because only 25 animals were used. It had obviously been considered in the nature of a pilot experiment and the controls consisted of the very large number of similar rats studied in other experiments over a period of many years in that laboratory. Also in several other laboratories there was an almost irresistible impulse to repeat the experiment with a larger number of animals. We succumbed to this temptation in our own institution.

Our study was started in December 1952. We attempted to duplicate Chaikoff's results using the same experimental conditions and, in addition, to make a comparison between the effects of radioactive iodine and an estimated equivalent external x-ray dose on the thyroid gland of the Long-Evans rat.

One hundred sixty-eight adult male rats divided approximately equally into three groups, i.e. 1) controls; 2) internally irradiated; and 3) externally irradiated, were examined periodically and sacrificed after two years.

The internally irradiated rats received a single intraperitoneal injection of 400 microcuries of I-131. The externally irradiated rats received a tissue dose of 900 roentgens in five days. This dose, which had been thought originally to be a calculated equivalent dose, turned out to be less than the I-131 dose, which often totally ablated the thyroid gland and caused myxedema in the animals. This resulted in observations of the effect of different dose levels which proved of considerable interest. After sacrifice of the 150 animals suitable for study, the thyroid gland together with the trachea was studied in serial section. There were 136 pituitary glands suitable for section. The results of the study in detail have been submitted for publication. In summary this reads:

A single intraperitoneal dose of 400 μc. of I-131 in this series of male Long-Evans rats maintained on a low-iodine diet induced symptoms of hypothyroidism and partial or almost complete ablation of the thyroid gland. Only one thyroid neoplasm was found in rats so treated. This is a benign follicular adenoma.

X-ray irradiation of similar rats, 900r in 300r doses, delivered in 5 days, did not induce hypothyroidism, nor was atrophy of the gland a striking feature in these animals. A large number of benign adenomas and similar tumors thought to be invasive, designated as the "solid" type, were found in these x-ray-treated rats.

A control series, not treated, was found to have neoplasms of these same types, but in smaller numbers, than the x-ray-treated animals.

Papillary and follicular carcinomas were not found in the controls but were found in the x-ray-treated rats. The only malignant tumor with metastases was found in an x-ray-treated rat, a follicular carcinoma with lymph node metastases.

The coexistence of multiple endocrine neoplasms was noted.

Benign and malignant neoplasms in other sites are reported.

Conclusions: In this small series, which has not been subjected to statistical analysis, there is suggestive evidence that in certain low dose ranges of x-ray - not high enough to cause atrophy of the thyroid - benign and malignant tumors develop in this strain of rats in high enough proportions to suggest that the radiation is a carcinogenic factor. That the only malignant papillary and follicular tumors - histologically different from the spontaneous neoplasms - were found in the x-ray-treated rats is in agreement with the more recent findings of Lindsay, Potter and Chaikoff that lower dose levels of I-131 irradiation were probably carcinogenic in similar animals.

Histochemistry research

The Histochemistry Research Laboratory, under the direction of Dr. Gabriel C. Godman, has engaged in the following investigations:

1. Correlated histochemical and electron microscopic studies of virus-cell relationships, with special reference to the intranuclear infection with certain adenoviruses. In collaboration with Drs. C. Morgan, C. Howe and H. Rose of the Department of Microbiology, investigation of the cytopathology of adenovirus infection has continued. It has been found that infection of cultured HeLa or FJ cells with adenovirus type 5 results in the formation of unusual, large intranuclear crystals which are composed of protein in addition to ordered arrays of DNA-containing virus. Some properties of this material have been studied. Using the correlative technique of electron microscopy

and histochemistry of the same objects, the stages of viral and crystal development in cells are being examined.

2. The constitution of the L.E. bodies of systemic lupus erythematosus, with particular reference to its protein moiety. Evidence has previously been presented showing that the DNA of the L.E. body, which is derived from the cell nucleus, is neither altered in state nor in relative amount as compared with normal nuclei, as had been widely supposed. Continued investigations, with Dr. A. D. Deitch, have shown that in the formation of the L.E. body from a nucleus a 2- to 3-fold augmentation of protein occurs. At the same time histones are apparently lost. The incurrent protein, which is presumed to be derived from lupus serum, appears to be bound to DNA. Interferometric microscopy, carried out with Dr. R. Rifkind of the Department of Medicine, has confirmed that a more than 2-fold increase of dry mass takes place in the L.E. transformation. The process of conversion has been followed visually by means of phase contrast microscopy.

3. The deoxyribonucleoprotein complex in the course of cellular differentiation in typical and atypical growth. With Dr. A. D. Deitch, the changes in the nuclear constituents and their mutual associations, as detected cytochemically, are under investigation. Cells of the lymphatic series, and differentiating osteogenic and chondrogenic cells are being studied.

4. Factors influencing the production of acid mucopolysaccharides by cells of osteogenic origin in tissue culture. With Mr. C. M. Morris, the influence of growth rate, cell numbers, altered physical conditions, medium composition, and chemical or hormonal agencies on quantitative production of acid mucopolysaccharide by strains of cells derived from membrane bone are under study. Efforts to obtain an accurate method of evaluating cell number in culture by means of micro-DNA determinations have been successful.

5. The influence of corticoids on the functional activity of strain "L" fibroblasts *in vitro*. Dr. H. Grossfeld of the Department of Medicine is studying the effects of cortisone and related steroids on the survival and metabolism of cells of strain "L" in tissue culture.

Cell physiology

Using tissue culture methods, the Laboratory for Cell Physiology under the direction of Dr. M. R. Murray is habitually occupied with a variety of basic research problems at the cellular level. Especially during the past year its function in post-doctoral training has been emphasized. On special fellowships from the U.S. Public Health Service, Professor F. Thomas Algard of Stanford University and Dr. Murray Bornstein of Mt. Sinai Hospital have been adapting tissue culture techniques, respectively, for investigating the histogenesis of hormone-dependent tumors in the hamster, and for the experimental study of the degenerative diseases of the nervous system. Dr. Louis R. Rey of the École Normale Superieure, Paris, spent several weeks as guest investigator learning methods of culturing differentiating nervous tissues.

In her continuing study of nutritional factors governing the development of the dorsal root ganglion complex, Mrs. Edith Peterson has been able with the aid of synthetic media to dissociate experimentally several elements which normally combine to produce the mature, or differentiated, neuron. Thus she has shown that myelin sheath formation is dependent not only on the orientation and development of the Schwann cells enveloping the fiber (credited by Geren with the major role in myelinogenesis) but also on the capacity of the neuron soma to produce Nissl substance.

In a direct chemical attack upon the problem of cytodifferentiation (in collaboration with Professor Erwin Chargaff) Mrs. Helena Benitez has been able to bring about, on a quantitative basis, a morphological

transformation in adult rat fibroblasts. When confronted in vitro with microsomes from homologous liver or kidney, these cells take on the appearance of cultured CNS tissue; when the agent is withdrawn, the fibroblasts gradually revert to their typical form. The relationship of RNA (as a major component of microsomes) to this change is being investigated.

In collaboration with Professor Dan Moore of Microbiology, Dr. Etienne Lasfargues has been engaged in a study of the milk factor for mouse mammary carcinoma. He has successfully propagated this carcinogenic agent in cultures of normal embryonic mammary glands, and is attempting by electron microscopy, correlated with various physical and chemical means, to identify and characterize it. It is hoped that by this correlated multiple approach, inconsistencies and contradictions which now characterize the literature of the milk factor may be resolved.

Employing his recently developed method for culturing adult mouse mammary tissue, Dr. Lasfargues has made ancillary observations which indicate that the lipid inclusions in the secreting mammary epithelium are of exogenous origin, deriving from adjacent adipose tissue, rather than being synthesized as lipid by the epithelial cells themselves.

With Professors Gellhorn and Hirschberg, Mrs. Ruth Eising has been continuing a comparative study of the effects of chemotherapeutic agents upon human and mouse gliomas in vivo and in vitro.

The mouse mammary carcinoma milk factor

The direction of the research efforts of Dr. C. D. Haagensen and his associates remains the same, namely, to identify and describe the factor in the milk of mice which produces breast cancer. Although we have continued to attack this problem by every available method, emphasis has shifted during the past year to the tissue culture method of study.

Mrs. Margaret Murray and Etienne Lasfargues have worked out satisfactory methods of growing the mouse breast tissue in tissue culture.

Electron microscope studies of cultures of both normal and neoplastic epithelium have revealed virus-like particles only in the neoplastic tissues. The quantity and prevalence of virus-like particles appears to be much greater in the cultures of cancerous tissue than in sections of the tumor itself. These particles, mostly localized in the intercellular spaces, are released into the culture medium where they may be seen in dispersed preparations. This suggests the possibility of producing large quantities of such particles and their isolation in order to test their specificity.

An antiserum prepared in the rabbit, by repeated inoculations of milk from tumor-agent-bearing mice, has a powerful destructive activity on epithelial cultures of neoplastic mammary tissue. This action is perceptibly milder on cultures of normal epithelium. Such *in vitro* tests are now being used to detect the specificity of various fractions isolated from the whole milk of agent-bearing mice.

Dr. Ernest Pollard, of Yale University, continues to collaborate in our project by deuteron bombardment of the milk preparations, using both untreated and versene-treated milk.

Tumor biology

Dr. Edward L. Howes has been studying certain biological and biochemical events during tumor growth:

Mucoproteins increase in the individual with cancer but it is not known whether this increase is a reaction to injury or the result of increased production of these substances by the tumor. Several are present.

The mucoproteins of 755 tumors are being isolated and measured quantitatively both for the amount and types of mucoproteins found in the tumor

tissue and in the blood of the host.

The relationship of the sprouting of blood vessels to the capacity of tumors to survive transplantation has been investigated in a general program studying the chemical changes that initiate or accompany the sprouting of capillaries. The tumors have been found to survive transplantation equally as well when placed in a field of growing capillaries (granulations) as in an area of delayed sprouting (cortisone effect). Moreover it has not been possible to isolate from a tumor any substance that promotes capillary growth or even a markedly proteolytic enzyme. Most of the large vessels seen about the transplanted tumors are veins. It has been shown, however, that transplantation requires plasma or whole blood.

Combination chemotherapy

Dr. Daniel M. Shapiro, together with Dr. L. S. Dietrich of the Department of Biochemistry and Dr. Maurice Shils of the Department of Surgery, has continued studies toward the two principal objectives of their research program: 1) to explore the feasibility of utilizing quantitative biochemical differences between normal and neoplastic cells as an approach to cancer chemotherapy; and 2) to study the possibility of eradicating neoplastic diseases by the simultaneous or sequential administration of combinations of chemical compounds selected upon this basis.

In systematically studying the B vitamins and their antimetabolites, we have been concerned most recently with niacin, and have noted carcinostatic activity with the niacin antagonist, 6-aminonicotinamide. This compound has been tested alone and in combination with 8-azaguanine and testosterone against the same tumor-host system (755 breast cancer in C57 mice).

The results with 6-aminonicotinamide (6-AN) alone range from inhibition of tumor growth, to stoppage of growth, to significant tumor regression

depending upon the dosage levels of 6-AN employed. A dose of 2.0 mg./kg., while allowing some tumor growth to take place, produced marked carcinostasis. Tumor growth was brought to a virtual standstill by the 3.0 mg./kg. dose. A dosage level of 4.0 mg./kg. caused significant tumor regression in male host animals, as evidenced by comparing the mean tumor weights of these groups with those of the sacrificed controls. In female animals, one out of three experiments showed significant tumor regression at the 4 mg./kg. dose. However, the other two experiments showed slight, but statistically insignificant regression of the tumors. There was virtually no mortality at any of these dosage levels. At the 2.0 mg./kg. dose there was only slight weight loss, but at dosage levels of 3.0 mg./kg. and above, host weight loss was pronounced.

The results obtained from combining 8-azaguanine and testosterone with 6-AN showed significant tumor regression at all dosage levels of 6-AN. However, it was noted that drug combinations including 6-AN at doses of 3.0 and 4.0 mg./kg. produced excessive host weight loss while those with 6-AN at a dose of 2.0 mg./kg. resulted in only minimal weight loss with little, if any, sacrifice in the degree of tumor regression. The ability of the double combination of 8-azaguanine and the 2.0 mg./kg. dose of 6-AN to produce tumor regression is of particular interest, since this level of 6-AN was capable of only retarding tumor growth when administered alone.

Reversal studies with nicotinamide were carried out with 6-AN to determine whether the observed mechanism of anti-tumor activity was related to its role as a vitamin antagonist. The carcinostatic effect of 6-AN was successfully reversed by the concomitant administration of nicotinamide. The vitamin showed no carcinostatic activity at the dose given and blocked the anti-tumor effect of 6-AN.

The optimal functioning of all cells, normal or malignant, appears

dependent upon metabolites and enzymes interacting in proper balance. It is, therefore, conceivable that this "balance" may be affected by an excess as well as a depletion of a cellular component. Thus, along with our studies of niacin antagonists, the effect of the metabolite, itself, on the growth of the tumor has been investigated. In the course of such studies with nicotinamide, it was found that high doses of the vitamin (600 mg./kg. twice daily) and above resulted in 75% carcinostasis. When nicotinamide at a dose of 600 mg./kg. twice daily was combined with 8-azaguanine and testosterone tumor growth was arrested. However, the addition of one more drug, deoxypyridoxine, to the above combination of compounds resulted in significant tumor regression.

Of particular interest, is the finding that not one of these compounds, nor any triple combination, regardless of dosage employed, was capable of producing more than inhibition or stoppage of tumor growth. It required all four compounds to effect tumor regression. On the basis of these data, the feasibility of complete tumor eradication by larger chemical combinations appears to gain added strength. This regressing combination furnishes clear negation of the contention of some investigators that the augmented anti-tumor effects obtained by combination chemotherapy are no better than would be obtained by giving one of the compounds in higher dosage; as noted above, only the simultaneous administration of all four compounds resulted in tumor regression.

Biochemical studies on 6-aminonicotinamide (6-AN) were divided into two general phases: 1) the search for co-factor analogues or other metabolic components containing the nicotinamide analogue, isolation and characterization of these compounds if found and the determination of their activity against pyridine nucleotide-dependent enzymes, in vitro; 2) the determination of the effects of in vivo administration of the drug on various

pyridine nucleotide-dependent enzymes.

6-AN analogues of DPN and TPN were isolated from tissues of animals administered 6-AN. These same pyridine nucleotide analogues were synthesized *in vitro* using purified enzymatic systems.

Enzymatic and chemical studies demonstrate that both compounds are inactive enzymatically, having no activity against lactic acid dehydrogenase, alcohol dehydrogenase or isocitric dehydrogenase. Furthermore, reactions involving additions of constituents at the 4 position of the pyridinium ion are either absent or fail to produce the characteristic fluorescence and accompanying change in absorption spectra which occurs following hydrogen, cyanide, acetone, or dihydroxyacetone addition to either DPN^+ or TPN^+. Therefore, we are tentatively assuming that the presence of an amino group at position 6 of the nicotinamide moiety of DPN^+ and TPN^+ prevents the reduction reactions which normally occur. Thus, the mechanism of action of this powerful niacin antagonist may be pinpointed.

The effect of 6-AN administration on tissue DPN^+ concentrations produced no significant changes in DPN^+ concentration of liver or tumor tissue. 6-AN did, however, interfere with the stimulating effect of high levels of nicotinamide upon DPN^+ synthesis in the liver tissue.

Studies of anaerobic glycolysis of liver and tumor tissue under conditions of 6-AN administration (8 mg./kg.) reveal no interference. However, assays of tumor lactic acid and 3-phosphoglyceraldehyde dehydrogenase activities of these same homogenates demonstrate that, although lactic acid dehydrogenase activity is normal, the activity of 3-phosphoglyceraldehyde dehydrogenase is halved. These are the only two DPN-dependent systems that participate in anaerobic glycolysis. It is interesting that 3-phosphoglyceraldehyde dehydrogenase is inhibited 50% by *in vivo* administration of 6-AN

and yet no effect on total anaerobic glycolysis is observed. This may mean that in this system (755 tumor) 3-phosphoglyceraldehyde dehydrogenase is not the limiting step, being present in such excess that loss of half of the original activity has no effect on the over-all production of lactic acid.

Three mitochondrial restricted enzymatic systems were studied in tumor and liver homogenates. They were α-ketoglutarate oxidase, β-hydroxybutyrate dehydrogenase, and pyruvate oxidase. In all cases, liver activity was unaltered by the chronic administration of 6-AN. Under identical conditions, tumor α-ketoglutarate oxidation was about 80-90% inhibited, tumor β-hydroxybutyrate dehydrogenase, 60% inhibited; and tumor pyruvate oxidase, 30% inhibited. These data again demonstrate the variable degree of inhibitory activity of a particular vitamin antagonist against the different vitamin-containing enzymes. They further demonstrate the need for defining quantitative biochemical differences at the vitamin-containing enzyme level, rather than in terms of total vitamin concentration.

If *in vivo* administration of 6-AN attacks the mitochondrial DPN-dependent systems dealing with oxidative phosphorylation, then shifts in the energy stores of the tumor should be evident. Accordingly, crude nucleotides were isolated from brain, liver and tumor of control and 6-AN-treated animals (8 mg./kg. x 4). These were chromatographed on paper in two dimensions. The individual nucleotides were cut out, eluted, characterized and quantitatively analyzed. No change appeared in the nucleotide pattern from treated brain and liver tissue as compared to control tissues. However, in the case of 755 tumor, adenosine triphosphate levels decreased by 70%; adenosine diphosphate levels by 40%; and adenosine monophosphate increased approximately the same amount that the adenosine polyphosphates decreased. Similar increases were quantitatively determined for guanosine monophosphate and cytidine

monophosphate. The following qualitative changes were observed: uridine monophosphate increased and guanosine di- and triphosphates decreased in concentration. Thus, tumor cells under these conditions present a picture of energy deprivation; namely decreases in polyphosphates of nucleotides and increases in nucleotide monophosphate.

In collaboration with Dr. M. Kligerman of the Department of Radiology, studies combining chemotherapy with simultaneously administered radiotherapy have been done. The chemicals employed were 6-mercaptopurine and 6-aminonicotinamide. When these compounds were introduced in the combined dosage method, percentage of cure in experimental groups of mice with breast cancer rose from 27 per cent with x-ray alone to 86 per cent.

Because these results suggest the desirability of clinical trial, the known information on 6-mercaptopurine was reviewed. This compound has been extensively explored by other investigators and has been found to have a dramatic effect upon numerous experimental tumors. However, when tested against human solid tumors, 6-MP has been found to have little or no effect. One possible explanation for these apparently different results may be found by examining the methodology employed. At the clinical level, compounds in the experimental stage are used against only the most advanced cases of human cancer, which means the compound must act upon extremely large tumor masses. In laboratory studies, treatment is usually begun from 1 to 7 days after tumor transplantation, which means tumor mass is relatively small. Therefore, 6-MP experiments were set up with treatment initiated on tumors of varying size in a gross attempt to correlate the wide range in tumor size found between laboratory and clinical studies.

Therapy was begun with 6-MP on 755 tumor-bearing animals 1, 8 and 15 days after transplantation. Results showed that complete tumor regressions

(% tumor "cures") were in inverse proportion to the day treatment was begun. Thus, on the large tumor masses (15 day), complete tumor regression was never produced, but moderate carcinostasis occurred.

These results suggested two possible explanations; that is, tumor age or tumor size. Therefore, experiments were conducted to study the age factor. The large tumor masses found at 15 days were cut back (by employing partial surgical excision) to approximate the size of an 8 day old transplant. 6-MP was administered to these latter groups of animals with the result that "cures" were produced, whereas no "cures" were produced in groups receiving either surgery or chemotherapy alone.

All of the above findings suggest that it may be possible, by employing surgery followed by a course of the appropriate antimetabolite therapy and radiotherapy, to save certain patients previously considered hopeless due to non-localized malignant disease. These possibilities are planned for clinical study in the near future.

Surgical pathology

Drs. L. Lane, J. Maim and R. Lattes have completed an analysis of the clinical-pathological correlations in our series of 117 malignant melanomas. This study shows that the overall 5-year survival of the cases receiving radical treatment in Presbyterian Hospital was 34 per cent. The most important single favorable histological feature was the tendency of the neoplastic cells to spread laterally rather than in depth (lateral junctional spread). The soundness of prophylactic regional lymph node dissection was proven by the demonstration of unsuspected histological metastases in the regional lymph nodes removed prophylactically in patients who had no palpable nodes and who survived at least five years post-operatively without evidence of

recurrence or metastasis. The best prognosis (60 to 70% 5-year "cures") was associated with the following combination: female sex; primary lesion less than 2 cm. in diameter; lateral junctional spread; no palpable regional lymph nodes; prophylactic lymph node dissection. This study will be published in detail.

Dr. R. Lattes has completed a clinico-pathological study of 84 cases of tumors of the thymus. The conclusions reached were: Most of the thymic tumors consist of an admixture of lymphoid and "epithelial" elements. The difference in the ratio between the two cell types, and the morphological variations among the epithelial cells, give rise to a great variety of histological patterns. When thymomas occur in patients with myasthenia gravis, more or less conspicuous sheets and nests of pale epithelial cells are found in about one-half of the cases. However, similar pale cells were seen also in non-myasthenia cases. Among the primary thymic tumors, non-associated with myasthenia gravis, there are two groups of lesions which are worthy of special mention; the so-called "granulomatous" thymoma, and the so-called "seminomatous" thymoma. It is possible that the former is a manifestation of Hodgkin's disease, and the latter a teratomatous lesion. On tissue culture study neither of these types of lesion has grown with the pattern typical of thymic tissue. Clinically, most of the thymic tumors are local problems: they may become inoperable because of local extension or pleural implants. Distant metastases are rare and occur in tumors which are otherwise atypical. Tissue culture is an invaluable tool in the study and differential diagnosis of mediastinal tumors of possible thymic origin.

Drs. R. Grinnell and N. Lane have completed an important clinico-pathological study on adenomatous polyp of the colon (about 1800 cases). This work, which cannot be summarized adequately in a short space, analyzes

in detail the significance of the adenomatous polyps and of the papillary adenomas as "precancerous" lesions, and the clinical implications of the histological finding of carcinoma in situ or invasive carcinoma in pedunculated and sessile polyps. This work will also be published in detail in the near future.

Drs. Lane and H. Dorfman have started a study of certain types of carcinoma of the stomach, in an effort to establish a correlation between the small carcinoma masquerading as benign ulcer, and the clinical course following sub-total gastrectomy. This work is in progress now.

Dr. Lane has completed and published an interesting study of an unusual variety of squamous cell carcinoma of the mucous membrane of the oral cavity and pharynx, which is associated with bulky masses of "pseudo-sarcomatous" appearance. The pathological study and especially the clinical course of these cases suggested that only the carcinomatous portion had truly malignant neoplastic properties. The interpretation of the nature of the "sarcomatous" component of these lesions is difficult. These cases do not fulfill the requirements for their classification as "carcinosarcoma" or "collision tumor". The suggestion is made that the "sarcomatous" component of these growths is probably not a malignant neoplastic tissue, and for this reason its tentative designation as "pseudosarcoma" is proposed.

Dr. Lattes, in collaboration with Professor H. Hamperl of Bonn, Germany, has completed and published an histological study on the differential diagnosis between non-chromaffin paraganglioma and the so-called alveolar soft part sarcoma (or organoid granular cell myoblastoma). With a modification of the Bodian stain suggested by Dr. Hamperl, it was shown that argyrophile granules are present in the cells of the non-chromaffin paraganglioma but not in those of the "organoid granular cell myoblastoma". This observation is of interest

in view of the suggestion by some authors that these two tumor types may be closely related.

Dr. Lattes, in collaboration with Dr. C. Grossi, has completed and published in detail a study of five cases of carcinoids of the stomach. It was felt that the unique biological behavior of these tumors, with slow progression even after regional metastases have occurred, makes them a clinical as well as a pathological entity. In evaluating long-term results after surgery for gastric cancers, these neoplasms should always be segregated from the larger and more malignant group of the other metastasizing epithelial tumors of the stomach.

Francis Delafield Hospital

The studies of Dr. Cushman D. Haagensen, assisted by Dr. Sami Obeid, American Cancer Society Fellow, on the spread of mammary carcinoma to the regional lymph nodes continues. A special emphasis is being placed on the value of the "apex of the axilla" biopsy as part of the triple biopsy. To date over 100 "apex of the axilla" biopsies have been done. Drs. Haagensen and Obeid have also succeeded in having both the Surgical Pathology Department and Presbyterian and Delafield Hospitals report the positive axillary lymph nodes in like manner so that in the future our results may be more accurately comparable for study.

Dr. Frederic P. Herter and Dr. Thomas V. Santulli are currently investigating the problem of suture line recurrence in colon carcinomas. They have been particularly interested in the influence of intestinal antibiotics on tumor cell implantation and have been conducting controlled experiments in rabbits using the Brown-Pearce carcinoma. This work will continue during the coming year. On the Gastro-intestinal Service at Delafield Hospital, as a reflection of this laboratory work, a controlled series of colon resections

have been done using preoperative antibiotic bowel preparation in alternate cases.

Dr. Clare A. Stein, American Cancer Society Fellow, has begun a very worthwhile study into the feeding of debilitated patients. She has studied fat absorption in a group of patients who have been placed on high-caloric protein diets in an attempt to reverse their cachexia. A Barron Food Pump has recently been acquired which permits giving a patient 2000 calories in a 2100 cc volume. This permits high-caloric feeding without diarrhea. This study of the nutrition of patients is of vast importance especially in cancer patients.

The Head and Neck Service under Dr. David Ju and Dr. Carl Feind has been carrying an increasing and heavy load in clinical material in the past year. We have noted a considerable decrease in the matter of postoperative lymphedema of the face following total glossectomy and bilateral neck dissection, by alteration in the incision used which helps preserve the dermal lymphatics.

In the past year lesions of the tonsillar fossa have been subjected to earlier surgical intervention. The results so far are encouraging. As is well known, this area has not often been subjected to surgical attack, as the results in the past have not been encouraging.

Dr. Grant Sanger has continued to study all of the women coming to the Delafield Hospital with carcinoma of the breast. With the cooperation of the rest of the surgical staff all breast lesions are accurately described on a special breast map. The details of the lesion and the patient's history have been recorded on the mammary carcinoma summary sheet, which Dr. Cushman D. Haagensen has been using for the past 18 years. Dr. Sanger is also continuing a survey of all the head and neck carcinomas admitted to Delafield since its

opening. The purpose of this study is to determine the reason for failure in this large group of patients, many of whom have been treated primarily in other hospitals in New York City.

Dr. Charles M. Findlay has for the past year been studying the action of tranquillizers on terminal carcinoma patients with and without pain. It appears that these drugs do not have a persistent effect and that they are not particularly valuable in the management of these patients. A study under Dr. Findlay's direction and with the assistance of the surgical staff is being carried out for Wyeth Laboratories on the use of Phenargan for postoperative medication of patients having endoscopic examination under local anesthesia. Preliminary findings show that the drug is of little use by itself, but that when it is combined with other common preoperative medications better results are obtained. A few patients have had tumors of the esophagus and bronchus injected with Pontamine sky blue dye. Subsequent supraclavicular fat pad biopsies were performed shortly after injection. The diffusion of the dye in these cases has been so inconstant that no conclusion can be reached at present.

Urology

At the Squier Urological Clinic, Dr. M. M. Mellcow completed a study on 185 tumors of the testis which was the subject of a paper entitled "Classification of Tumors of the Testis". A working classification was included which was based on the premise that testicular tumors arise from one of three cell types: interstitial, Sertoli and germinal (spermatogones or spermatocytes). Tumors arising from the first two were rare, whereas those from the last comprised the bulk. Germinal tumors were of two varieties: a) Seminomas (dysgerminomas), which may be looked upon as "sterile" tumors because the component cells hold on to, or have lost the potentiality of forming three germ layers. They merely repeat their own pattern as they multiply. b) Embryomatous and teratomatous tumors, which may be looked upon as "fertile" tumors in that the potentiality for germ layer formation has been unlocked from the nuclei of their component cells. This results in an attempt to simulate events that are set in motion when an ovum is fertilized: cell division leading up to the formation of a blastula with trophoblasts, then a splitting into three germ layers which in turn form organs and tissues and which are then organized into systems that comprise the completed being (fetus). In these tumors there probably is first a rapid formation of evanescent blastulas. Embryoma, usually in association with trophoblasts (chorioncarcinoma); then a breaking into a hodge-podge of 3 germ layer elements with a predominance of one or more layers: embryonal carcinoma. Then there is a partial attempt for some portions to differentiate into tissues or parts of organs while the remainder continue as an embryonal stroma (teratocarcinoma); and finally there may occur, rarely, almost complete differentiation into a scrambled mass of tissues and parts of organs (adult teratoma). It is all a moving picture process and the rate of

development varies with each patient and the findings depend on the stage when the tumor is discovered and excised. Combinations can occur because some areas within a tumor may undergo changes faster than other areas. It is to be expected that

1. Embryoma is an unstable, temporary stage and therefore rare,

2. Choriocarcinoma is also rare. It tends to metastasize early and kill.

3. Embryonal carcinoma, and

4. Teratocarcinoma are the common tumors and

5. Adult teratoma is benign and rare.

The majority of nongerminal tumors were of the interstitial variety. The granulosa cell tumor in our series was an interesting pathological finding. The majority of the secondary tumors were lymphomas and cancers of a similar origin. Lymphomas metastasizing to the testis were sometimes mistaken both clinically and pathologically for seminoma, particularly when the presenting symptom was a testicular swelling. A correct differential diagnosis in these lesions is important, in view of the difference in prognosis. Primary testicular tumors were rare in children. There were no seminomas in this group.

Dr. Melicow completed a comprehensive report on bladder tumors, their classification and results of treatment. It was based on a clinico-pathological review from the files of uropathology of the Squier Clinic, the Department of Pathology, Columbia University, Surgical Pathology of the Presbyterian Hospital and the pathological material from Sloane Hospital and Babies' Hospital. Ninety-five per cent of 914 cases of primary bladder tumors developed from the urothelial lining and over 5% (42 cases) arose from the stroma. Of the former 81.1% were papillary urothelial tumors and 7% were

non-papillary; about 11.4% were metaplastic (squamous cell and glandular) and 0.5% were glandular of urachal or subtrigonal origin. Of the papillary tumors 92 or 12.4% were papillomas; 187 or 25.3% were grade I carcinomas; 182 or 24.7% were grade II carcinomas; 150 or 20.2% were grade III carcinomas and 130 or 17.5% were grade IV carcinomas. Fifteen of the stromal tumors were benign and 25 were malignant. Fifteen of the latter belonged to the myosarcoma group.

The problem of therapy of vesical cancer was discussed in the light of four alternate objectives which motivate the attack on malignancy: a) the ideal - destroy the cancer and preserve anatomy and function; b) the practical - destroy the cancer with moderate sacrifice of anatomy and function; c) the radical attempt to destroy an extensive cancer by considerable sacrifice of anatomy and function; and d) the palliative attempt to maintain life with relative comfort in patients with inoperable malignancies. Underscoring all treatment should be the search for the carcinogenic agent(s) and for the factors which aid growth, accelerate spread and induce recurrence; and for those which have an opposite effect.

The following unique features of bladder cancer made adequate treatment difficult:

a) All therapeutic procedures violated a major dictum in cancer therapy, i.e. not to operate in a cancer-contaminated field. This was probably a major factor in "seeding" cancer cells and the cause of many "recurrences".

b) The severity of bladder cancer varied not only with the grade but also with the site, size, shape and depth of tumors. No one method of treatment was applicable to all.

c) Microscopic foci of neoplasia, invisible to the naked eye, were present in some bladders at the time of destruction or removal of a large tumor.

Subsequently the foci came of age and the patient returned with more tumor(s).

a) An unknown carcinogenic agent probably continued to act on the vulnerable urothelium even after adequate removal of a tumor and thus led to the development of a new one.

Transurethral fulguration or resection of a bladder tumor fulfilled objective # 1. The procedure, however, was limited to those neoplasms which were readily accessible and which could be ablated at one sitting. The papillary tumors with narrow pedicles responded well to this modality. Neoplasms which had penetrated the base were treated more adequately wtih the resectoscope but it was often difficult to gauge the depth to which the tumor had reached.

The great frequency of multiple recurrences following transurethral fulguration suggested the possibility of seeding resulting from manipulations by the instrument and/or (more probably) the continued action of a carcinogenic agent(s) acting on vulnerable urothelium.

Suprapubic segmental resection met objective # 2 in the management of large, broad, sessile growths arising from mobile portions of the bladder. Recurrences, however, were frequent and there was a great incidence of abdominal scar involvement by tumor due probably to seeding at the time of operation. The use of strong chemicals to destroy free cancer cells was ineffective in preventing recurrences. It was not possible to kill all the cells and in the meantime the injured surface apparently acted as a more receptive field for implants. Scrupulous care in handling the neoplasm during operation and protecting the abdominal wall is indicated. Change of towels, gloves and instruments after completion of the resection is advisable.

Large, penetrating tumors, particularly in fixed regions of the bladder, were often treated effectively with radium needles inserted via the suprapubic

route. The precautions mentioned under the previous paragraph also apply here. Postoperative morbidity from inflammation, infection, ulceration and calcareous debris was a serious complication. Thus objective # 2 was only partly met since considerable functional disturbance followed this form of therapy.

Cystectomy and ureteral transplantation were carried out in patients with extensive surface involvement, in those with lesions located on the trigone and/or bladder neck, and as a last resort on patients who, in spite of numerous previous treatments (fulgurations, radium or segmental resection), continued to return with more and more tumors.

Theoretically, complete removal of the entire cancer-bearing area and of all the vulnerable urothelium should have been the ideal procedure in a disease which has such great tendency to recur. It met objective #3. Bladder function was lost, but a cloaca was substituted. Unfortunately the results were frequently unsatisfactory. Electrolytic disturbances, acidosis and above all ureteral obstruction, hydroureter, hydronephrosis and pyelonephritis were frequent and serious complications. Life with comfort was prolonged only in a few cases.

If it were possible to tell <u>in advance</u> which case would return again with recurrence(s) following other forms of therapy, then <u>cystectomy performed before</u> infection, mural extension, renal damage, constitutional deterioration could set in, might save much pain and trouble to both patient and doctor. Unfortunately, there is no way at present of determining this in advance.

Extensive mutilating operations (total cystectomy, enteration, etc.) in patients with deep-seated invasive tumors increased debility, destroyed personality and did not significantly lengthen postoperative life span. Palliative therapy (objective # 4) was applicable in such cases and consisted

in measures to relieve pain, lessen or remove obstruction and combat infection.

In conclusion, it is not possible in thepresent state of our knowledge to be dogmatic with regard to the superiority of one form of therapy over another. But it is important to know the nature of the enemy, to understand the problem, to see the indications, effectiveness and limitations of all the modalities of therapy - - that is half the battle; and it was with this objective in mind that the review was made.

An article by Dr. Melicow on "Hibernating Fat and Pheochromocytoma" was published. This interesting observation of brown fat in association with pheochromocytoma is being studied further in our laboratory.

Dr. M. M. Melicow and Dr. A. C. Uson are at present engaged in the following studies:

1) A correlation of gross uropathology with urography with the object of elucidating principles in diagnosis which will aid in earlier recognition of the various neoplastic lesions of the urinary tract. The entire subject will be presented in a book entitled "Integrated Urography and Gross Uropathology".

2) A clinico-pathological evaluation of genito-urinary malignancies including those of the adrenal gland and retroperitoneum. The objectives are to obtain a satisfactory pathological classification, to present suggestions for earlier clinical recognition, to improve surgical procedures and follow-up control.

3) A program of further studies into the pathology of vesical neoplasms, particularly in regard to newer special staining techniques.

4) A clinico-pathological report and review titled "Gonadal Neoplasms in Intersexes".

5) A clinico-pathological report (together with Drs. A. Damon

and D. Kolub) on the combination of renal neoplasms with polycythemia.

6) A study and report on the cellular changes in prostatic acini in association with infarcts, estrogen therapy, etc.

Under the direction of Dr. Perry B. Hudson, basic cancer research in the fields of steroid biochemistry, enzymology and nucleic acid chemistry has been continued. The group includes, at the professional level, Drs. T. Duane Price, Michael E. Lombardo and Rosemarie Bauer, working in the cancer laboratories of the College of Physicians and Surgeons and at the Francis Delafield Hospital.

In an attempt to find a correlation between values for urinary estrogens and the response to hormonal depletion operations in mammary cancer patients, samples of urine were assayed in nine patients before such surgery, after the ovaries and one adrenal gland had been removed, and after completion of bilateral ovariectomy and bilateral adrenalectomy. The results of this experience substantially support the contention made by Thomas Dao in 1953 that patients having a "high" urinary estrogen level may be expected to respond favorably to adrenalectomy.

Eight other patients have been studied by means of assay of adrenal venous blood in addition to urine. In each of these patients, both ovaries and the right adrenal had been removed. After a period of time to insure depletion of other sources of endogenous steroidal substances, samples of adrenal venous blood were collected by cannulation at the time of left adrenalectomy. Exogenous steroidal contaminants were eliminated by withholding cortisone acetate and other supportive measures involving the use of steroids until collection of the blood was completed. Preliminary bioassay studies indicate the presence of estrogenic material in human adrenal venous blood. An attempt at chemical characterization of these estrogenic substances

is now in progress.

Incubated samples from adrenal glands obtained from mammary cancer patients were extracted and chromatographed. These studies have demonstrated the 11β-hydroxylation, 17α-hydroxylation, and 21-hydroxylation of the steroid molecule by human adrenal tissue *in vitro*, evidenced by the conversion of progesterone, 17α-hydroxyprogesterone and 11-deoxycortisol to cortisol.

It has been conclusively demonstrated, using both radioactive and non-radioactive progesterone, that normal human testicular tissue can utilize this steroid hormone as a precursor for androgen biosynthesis. The mechanism appears to be one of hydroxylation of the C_{17} position and reduction of C_{20} ketone with the subsequent removal of the side chain as a one- or two-carbon unit. The intermediates isolated and identified were 17α-hydroxyprogesterone and a C_{17}, C_{20} glycol. The authentic androgen isolated was testosterone.

C^{14}-labeled steroids have been used in experiments to investigate the role played by the bile in the excretion of steroid metabolites. The bile and urine of dogs with surgically produced biliary fistulas were extracted and fractionated, and the radioactivity of the fractions quantitatively determined. Preliminary results indicate that the hepatic and biliary participation in the metabolism of steroidal substances is significant.

Methods have been developed for the critical quantitative evaluation of the microgram quantities of nucleotides available in the acid-soluble fraction, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) of small samples of human tissue, and detailed studies of absolute turnover rates done. Nucleotide composition and metabolism of normal human tissues have been found to be roughly but not exactly the same as those of the malignant tissues studied.

Special technical problems in investigation of the mechanism of nucleic acid biosynthesis in surviving human cells were found to be concerned with

negligible permeability of cell walls to mononucleotides, and rapid reversible side reactions of the mononucleotides inside the cells.

Isotopic tracer experiments with small-molecule precursors of nucleic acids have included study of reactions which constitute paths of entry and those which may be classified as side reactions.

A new assay method of tripeptidase was developed, and used to assay the enzyme when its level is elevated, as in certain pathological conditions, like cancer and liver disease. Contrary to previous theory that high levels of tripeptidase were indicative of cancer alone, it was discovered that in patients with cirrhosis of the liver, metastatic disease to the liver and other disorders which produced biochemically demonstrated hepatic impairment, the tripeptidase values were also abnormal.

A method of purifying solubilized uricase was developed during investigations into the potential enzymatic control of uric acid metabolism. In animal experiments, about 50% demonstrated uricolytic response to the enzyme. A clinical trial has also been made in two patients. Since the presence in the circulation of excess uric acid in some cancer patients undergoing x-ray or chemical therapy is well known, and as the rate and total amount of destruction of cancerous tissue by x-radiation increases with the development of new methods and as chemotherapy becomes more and more effective, the problem of removal of such breakdown products from nucleic acids will increase in importance.

In a study of acid phosphatases, an almost pure form of a phosphomonoesterase was isolated from yeast, and its stability characteristics determined. The enzymes present in the human erythrocyte were also the subject of investigation, and a method was developed for isolating an almost pure form of a purine nucleoside phosphorylase; its specific properties were described.

In the course of the study of the biological behavior of prostatic cancer, 710 biopsies of the prostate gland have been performed in one series of patients at the Francis Delafield Hospital since 1951. The incidence of unsuspected prostatic cancer has been found to be approximately 12%, confirming earlier autopsy studies made elsewhere. The system of follow-up has yielded a rate of 80%. The statistical data being accumulated also will permit evaluation of various forms of therapy in malignant disease. These include palliative as well as curative methods of treatment.

A method of urinary diversion has been developed, in which a segment of terminal ileum is employed as a "physiological valve" between the ureter and sigmoid to relieve back pressure upon the ureter by utilizing the unidirectional peristaltic action inherent in this portion of the ileum. After a series performed upon dogs, ureteroileosigmoidostomy has been employed in five humans with encouraging but equivocal results.

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